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PHYSIOLOGY

CONDITIONED REFLEXES IN DECORTICATE RABBITS

O. A. Krylov

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(Received April 26, 1958. Presented by Active Member of the AMN SSSR P. S. Kupalov)

There is now no doubt that in the higher animals, at any rate, conditioned reflexes are developed in the cerebral cortex. However, the question has frequently been raised as to whether the cortex is the only area in which such reflex connections can be established.

On this point, I. P. Pavlov said that "it is possible that at certain times and under certain conditions conditioned reflexes may be formed in parts of the brain outside the cerebral hemispheres. It is not possible to make a categorical statement, because all our classifications and laws are to some extent provisional and apply only to a particular time, to particular methods, and to the material at hand" [12].

It has frequently been pointed out [1, 2, 10, and others] that conditioned reflexes may be formed in decorticate guinea pigs, cats, and dogs. It is true that, as several investigators have pointed out, these reflexes differ considerably from those formed in intact animals. Thus, N. F. Popov [13], working on decorticate dogs, was able to develop a positive response to a metronome after presenting it more than 200 times in combination with an electric shock. However, the raising of the foot to the sound of the metronome did not take the form of an isolated reflex, but there was a generalized response in the form of disorderly movements not only of the leg but of all the other legs and the rest of the body.

According to E. Kuller and F. Mettler [14], and F. Mettler, S. Mettler, and E. Kuller [15], in response to the conditioned stimulus the decorticate animal merely raised the whole of the hind portion of the body from time to time, so bearing the whole of its weight on its front legs, i.e., it simply made a jumping movement.

S. I. Lebedinskaya and S. L. Rozental' [10], when forming a feeding conditioned reflex by I. P. Pavlov's classical secretory method, found no essential difference between decorticate and normal animals in the rate of formation or the character of the conditioned reflexes.

Evolutionary physiology confirms the possibility that conditioned reflexes may develop in decorticate animals. It is known that the lower and less highly organized animals which have no cortex are nevertheless capable of forming conditioned reflexes, i.e., temporary connections [5, 6, 7, 8, 9, 11, and others].

However, in the higher animals, the ability of the subcortical structures to form temporary connections has not been sufficiently studied. In some animals, such as the rabbit, no investigations of this type have been made. The present work on decorticate rabbits, represents an attempt to determine the method of development and the changes occurring in conditioned defensive reflexes involving shaking off the stimulus.

METHODS

The experiments were performed on 10 rabbits, of which 2 were used as control and 8 were decorticate. The cortex was removed from the upper and lateral areas of the cerebral hemispheres. Parts of the cortical areas

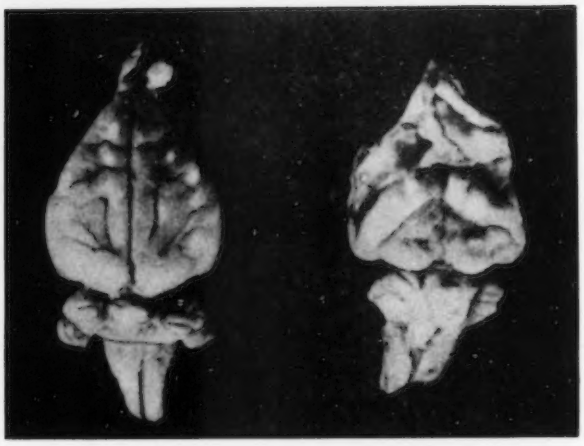

Fig. 1. On left, brain of normal rabbit, and on right, that of decorticate animal (seen from above)

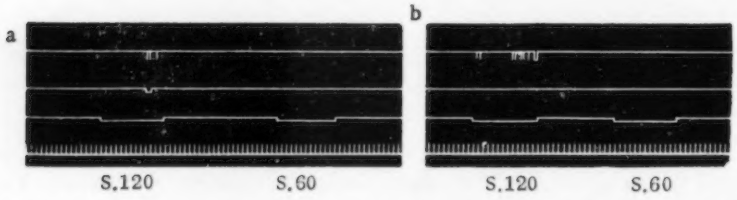

Fig. 2. First experiment (2 1/2 months after decortication) using a conditioned and a differentiated stimulus in (a) a decorticate rabbit, and (b) in a control animal.
Curves, from above downwards: "shaking off" movements; time of action of unconditioned stimulus; time of action of conditioned stimulus; time marker (1 second); s.120–conditioned positive signal, s.60-differentiation.

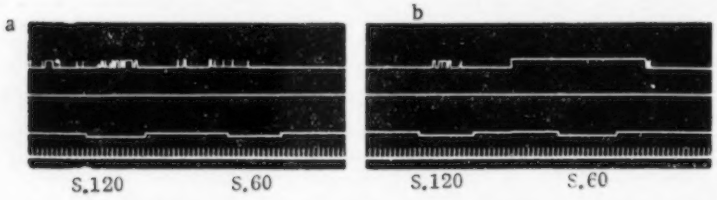

Fig. 3. Conditioned reflex shaking and its differentiation in decorticate rabbits No. 3 (a) and 4 (b). Notation as in Fig. 2.

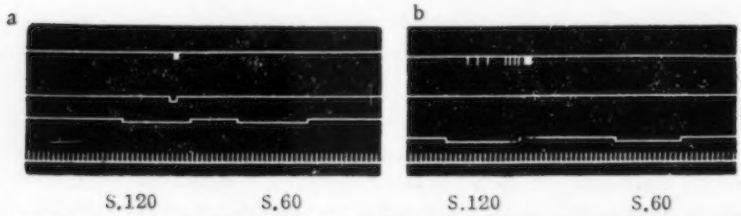

Fig. 4. Conditioned "shaking off" reflexes in a) decorticate and b) control rabbits after a 9-month interval. Notation as in Fig. 2.

adjacent to the cerebellum, and at the base of the brain, were left intact. Of the 8 operated animals, 4 perished a few days after the operation, 2 lived for half a year, while the remaining 2 survived for more than $1\frac{1}{2}$ years before being killed for histological examination.

Examination with the naked eye revealed that 60-80% of the cortex had been removed; the parietal areas had been removed completely, the only parts of the frontal areas remaining were those in the neighborhood of the olfactory bulbs and the base of the brain. The temporal portions remaining were found only at the base of the brain, where a very thin portion remained. Small parts of the occipital areas were preserved close to the base of the brain and the cerebellum. The lateral ventricles were greatly enlarged and opened (Fig. 1). Before and after decortication, the conditioned motor defensive "shaking off" reflex was developed using the method of A. A. Volokhov and G. A. Obraztsova [4].

Conditioned stimuli consisting of a flickering light or the intermittent sound of a bell ringing at 120 strokes per minute were given for 10 seconds at each stimulus. The stimulating electrodes were placed on the outer ear.

The strength of the current was somewhat above threshold. The induced "shaking off" movement was recorded using a mercury interruptor attached to the parietal region of the skull by "Mendeleev's" cement. Electrical reinforcement was used only when no shaking occurred in response to the conditioned stimulus; if the shaking did occur, no reinforcement was given. A bell operating at 60 strokes per minute was used as a differentiated stimulus.

The animal was allowed to move freely about the compartment during the test. The conditioned stimuli were applied every 2 minutes. They were given in the following sequence: light 120+, bell 120+ , and bell 60-, bell 120+, light 120+, bell 120+, bell 60l, bell 120+. The "shaking off" movements, the time of application of the conditioned and the unconditioned stimuli, and the time marker were all recorded on the kymograph.

RESULTS

In the control experiment, the conditioned reflex was usually established by the second or third day, or sometimes not until the fourth. At first it took the form of a generalized motor reaction in which the animal ran away. Shaking occurred only in response to the unconditioned stimulus.

From the 10-15th day onwards, in addition to the simple evasive response, a conditioned reflex "shaking off" reaction also developed. In most cases, and in all the subsequent experiments, this conditioned reflex "shaking off" occurred at the same time as the animal made its escape. Only in rabbits Nos. 5 and 7 was the response confined to the "shaking off" movements.

The differential stimuli were applied on the 13th day, the first response followed on the 15-17th day, and became stable by the 21-27th day.

The positive and negative conditioned reflexes were "learnt" very thoroughly. In all, 70 experiments were carried out on each rabbit, i.e., more than 400 positive and over 100 negative conditioned reflexes were evoked. Subsequently, 8 animals (Nos. 3-10) were decorticated. Two (Nos. 1 and 2) were kept as controls.

For the first few days after the operation, the rabbits sat motionless. They were artificially fed by placing of carrot in the mouth, and they could be made to feed only in this way. Later, those which survived (Nos. 3, 4, 7,and 9) began to move about, and fed themselves.

In order to avoid any effect on the conditioned reflexes from purely traumatic causes, the experiments were not resumed until $2\frac{1}{2}$ months after the operation. In the operated animals, the conditioned reflexes had disappeared entirely, while in the control animals both positive and negative reflexes were well preserved (Fig. 2).

Two and a half months after the operation, decorticate rabbit No. 3 became even more mobile than before, and more mobile than the controls. The first "shaking off" conditioned reflex appeared in it on the 3rd day of the experiment (after the 18th combined application of the stimuli) and became fairly stable from the 5-7th day onwards (after the 35-45th combined stimulus).

From the times of appearance of the conditioned "shaking off" reflexes, it would appear that the conditioned reflexes were not restored, but were built up again from scratch.

The "shaking off" reaction in Rabbit No. 3 was better shown than in the intact animals. While in the

compartment, it was moving about the whole time (particularly during the first month of the experiments); as a rule it ran in a circle, and frequently made the shaking response in between the signals. When the signal was applied, the shaking became much more vigorous (Fig. 3, a).

Rabbit No. 4 was quieter and reacted less frequently between signals (see Fig. 3, b). In it too, the "shaking off" reaction was better shown than in the controls; the general escape reaction was comparatively weak. Rabbits Nos. 7 and 9 moved about very little after the operation. Usually they sat in the tightly hunched up position. Conditioned reflexes were elaborated only with great difficulty, and did not occur until 50-70 combined stimuli had been given. The conditioned "shaking off" reflex sometimes occurred very violently. However, the responses were very unstable, and occurred only in occasional experiments. There was usually no conditioned escape reaction. They died half a year after the operation, having developed large trophic abscesses on the legs.

Rabbits Nos. 3 and 4 lived for over a year, and remained in good condition. They showed not only a well-developed "shaking off" reflex, but were capable of responding to the differentiated stimuli, though the power to do so developed much more slowly and was somewhat less stable than in the controls.

In the control animals a 9-month break had practically no effect on their conditioned reflexes; in the decorticate group, the "shaking off" reflex disappeared completely (Fig. 4). The conditioned stimulus caused only an orienting and search reflex.

It is therefore possible to develop good positive and negative conditioned reflexes in decorticate rabbits, but they are less stable than those in intact animals.

In spite of the fact that the possibility of forming conditioned reflexes in decorticate animals may now be considered to be established, many workers ignore the formation of temporary connections and the development of conditioned reflexes in subcortical areas. Some, for instance N. F. Popov [13], consider that the responses of decorticate animals to a conditioned stimulus should not be regarded as conditioned reflexes because of their crude nature and diffuse appearance. It is not easy to accept this view, since in Popov's own experiments (using an unconditioned pain stimulus to a dog's foot), the response to conditioned stimulation was just as precise as to the unconditioned stimulus.

In decorticate animals, reflexes which have been integrated at the lower levels of the central nervous system remain unchanged, or , more precisely, less changed. Conditioned reflexes developed from comparatively simple unconditioned reflexes are almost the same in their end result and in their method of development as those in normal animals; such responses include autonomic reflexes, such as salivary secretion in response to a food stimulus [10].

It is known that decorticate rabbits shake themselves much more vigorously than normals [3]. It therefore seems entirely natural that in our experiments the conditioned reflex shaking movements in the decorticate group were shown more definitely than in the controls; the latter had a greater tendency simply to run away, so that it was very difficult to develop the conditioned "shaking off" reflex, which could only gradually be made to replace the generalized escape reaction. It is interesting that young rabbits show first a better unconditioned and then a better conditioned "shaking off" reflex than do adults, and in this way they show some resemblance to decorticate preparations [3].

Nevertheless, we do not consider that differences in the way in which conditioned reflexes develop in normal and decorticate rabbits consist only in differences in their degree of complexity or perfection, or in the level of their integration in the central nervous system. In the decorticate preparation, the conditioned reflex connection is less well made. As had been already pointed out, this weakness is shown by an instability of the differentiated inhibition and in the less stable positive conditioned reflexes, the long break in the experiments eliminating all the connections which had been formed, while no such effect occurred in the normal group.

The conclusion is that both positive and negative conditioned reflexes may be elaborated in the subcortical region of decorticate animals, but that the reflexes are less stable.

SUMMARY

Experiments were performed on rabbits using the conditioned reflex method developed by A. A. Volokhov and G. A. Obraztsova; these showed that in decorticate rabbits it is possible to develop defensive reflexes which

consist of making "shaking off" movements, and that differentiations may be developed (the conditioned defensive signal was either the sound of a bell, or a light flickering at 120 times per minute; a bell striking 60 times per minute served as the differential signal). However, in decorticate rabbits the positive and negative conditioned reflexes are less stable, and become extinct when the experiments are stopped for 9 months, whereas in the intact animals the reflexes are preserved. Both the conditioned and unconditioned "shaking off" reflex is better developed in decorticate than in intact rabbits, the latter showing a greater tendency simply to run away.

In this respect the behavior of the decorticate rabbit is more like that of the young animals.

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CHANGES IN CARDIAC CONDITIONED REFLEXES IN DECORTICATE CATS

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(Received April 22, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

There are numerous references [6] to the fact that decorticate dogs are capable of developing defensive conditioned responses. The same reaction has been developed in decorticate cats [5]. Recently it has been possible to develop and to study feeding as well as defensive conditioned reflexes in such animals [1, 2]. Further, it has also been possible to elicit conditioned reflexes associated with respiration [4].

The aim of the present work has been to form temporary connections affecting cardiac activity in decorticate cats.

METHOD

Three cats were used. In 2, Blanche and Panther, the cortex of the cerebral hemispheres (neocortex) was removed in two stages with an interval of one month between the operations. In a third cat, Pepper, the formation of cardiac conditioned reflexes was begun before decortication was complete, at the stage when the right hemisphere had been removed. Extirpation was made by the method described by N. Yu. Belenkov [3]. Work on developing the conditioned reflexes was begun one month after the last operation.

A 1000 c/s sound was used as a conditioned stimulus. Ammonia vapor was the unconditioned stimulus, and was conveyed from the bottle to the animal's nose by a glass funnel. The conditioned stimulus was applied for 5 seconds. For the next 5 seconds the sound and the ammonia vapor were both given together. In each experiment, 5-6 combined applications were given at intervals of 3-5 minutes.

The electrocardiogram was recorded using an EKP-4 apparatus. Respiratory movements were recorded simultaneously on the kymograph.

RESULTS

It is known that ammonia vapor slows the heart beat. Thus, for instance, in Blanche the normal heart rate was 171 per minute, but inhalation of ammonia vapor lowered the rate to 136 (Fig. 1, a). In Panther, the normal heart rate was 222, and after ammonia – 169.

A check was made that the conditioned stimulus itself (the sound) caused no change in the electrocardiogram.

It was found that it was possible to establish a conditioned cardiac reflex in the decorticate preparations. The first conditioned reflex bradycardia was observed in Blanche at the 30th application of the stimuli, and in Panther the same effect occurred on the 20th occasion. Reinforcement of the reflex was applied after 90-100 applications. In Blanche, the rate was reduced by from 16 to 30 beats per minute (Fig. 1, b), and in Panther it fell from 12 to 20 beats per minute. There was also a conditioned reflex reduction in the amplitude of the respiratory movements.

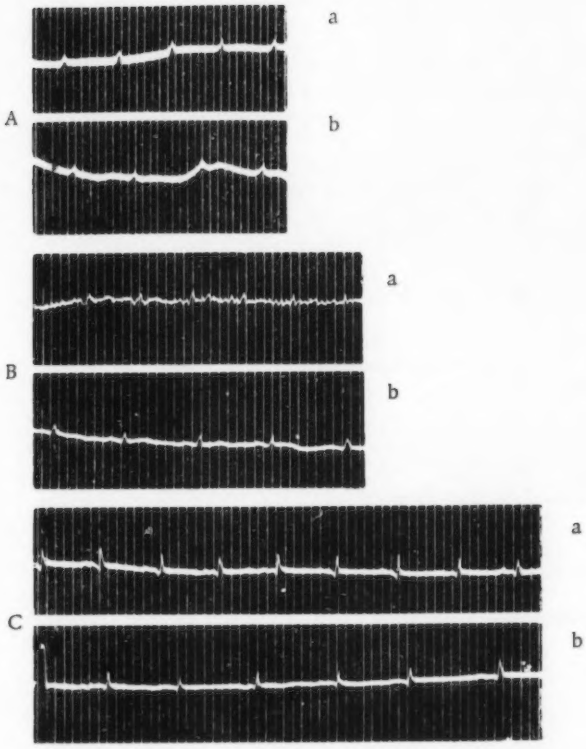

Fig. 1. Cat electrocardiograms. A) effect of ammonia vapor on the heart in Blanche. a) Normal; b) during inspiration of ammonia vapor. B) Conditioned reflex reduction in the heart rate in Blanche. a) Normal; b) during the action of the conditioned stimulus (1000 c/s sound). C) Conditioned reflex response to the first application of the conditioned stimulus after complete decortication, cat Pepper. a) Normal; b) during the action of the conditioned stimulus.

The temporary connections formed were very stable. After a three month interval, the conditioned reflex bradycardia could still be elicited. Thus in Blanche the reduction was then 9 beats per minute, and in Panther 12. The original extent of the reduction was then restored after a few further stimuli.

The conditioned reflexes which had been developed, were then extinguished by the usual methods. In Blanche, the first indications of extinction occurred after 16 applications of the conditioned stimulus without auditory reinforcement, and after 40-46 such stimulations there was no response at all to the conditioned signal (Fig. 2). The conditioned respiratory reflex disappeared at the same time as the cardiac. Conditioned reflexes inhibited in this way were easily restored. On the day following the extinction, the first application of the conditioned signal induced a bradycardia.

Having shown that cardiac conditioned reflexes could be established in Blanche, we then attempted a differentiation. The negative stimulus was a 500 c/s sound, and the positive stimulus a sound at 1000 c/s. The positive and negative signals were presented alternately, 7 negative and 5 positive stimuli being given in each experiment. After 14 days, during which 106 negative stimuli were given, differentiation was obtained, which was shown by the fact that there was no change in heart rate during the action of the unreinforced negative signal. The differentiation also occurred in the respiratory responses.

Experiments were also carried out on the hemidecorticate cat Pepper. Conditioned reflex bradycardia developed at the 5th combined application of the stimuli, and had become much stronger by the 15th. The reflex was very stable. The conditioned reflex bradycardia could still be elicited after a three-month interval.

After 136 applications of the combined stimuli, the second (left) hemisphere was removed. One month after this operation, the animal was again tested. It was found that the previously developed temporary connection affecting the heartbeat was still present, and even at the first application of the conditioned stimulus, a typical conditioned reflex bradycardia developed, the heart rate falling from 187 to 136 beats per minute (Fig. 1, c).

Naked eye postmortem examination of the brains of the two cats (Panther and Pepper) showed that the whole of the cerebral cortex had been removed except for a small portion (part of the gyrus proreus) in Pepper.

SUMMARY

Experiments were performed on 3 decorticate cats. Cardiac conditioned reflexes were developed by applying a combination of a 1000 c/s sound and the smell of ammonia vapor twenty or thirty times in succession. The reflex consisted in a decreased frequency of heartbeat in response to the conditioned signal alone. The elaborated conditioned reflexes were stable, and were maintained even after a three-month interval. It was possible to establish a crude differential response in the decorticate animals. In one hemidecorticate animal, the conditioned reflex established was maintained when the second hemisphere was removed.

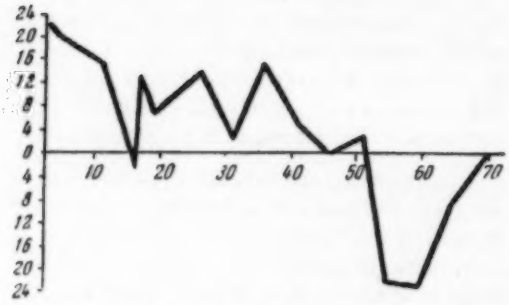

Fig. 2. Extinction of the cardiac conditioned reflex. The figures on the abscissa indicate the number of times the stimulus has been applied. The ordinate indicates the difference between the heart rate before and after conditioned stimulation. Figures above the line indicate slowing, and those below a speeding up.

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*In Russian

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CHANGE WITH DOSE OF THE CARDIOVASCULAR AND RESPIRATORY RESPONSES TO CHEMICAL STIMULATION OF THE SMALL INTESTINE RECEPTORS

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Cardiovascular and respiratory unconditioned interoceptor reflexes can be divided into two groups which appear at first sight to be of opposite kinds. The first group includes reflexes occurring on stimulation of the mechanoceptors of the aortic and sinocarotid zones, as well as those induced by stimulation of cardiac and pulmonary receptors, and which together are known as constituting the "phenomenon of Bezold'd-Yarish". The afferent pathway of the reflexes just described is along the nerves running to the medulla. When eliciting these reflexes, most workers observed a reduction in cardiovascular and respiratory activity. The second group consists of the reflexes which have been studied in detail by V. N. Chernigovskii and his coworkers [5-7] and which are evoked by stimulating various interoceptors of the abdominal cavity; the afferent fibers concerned enter various segments of the spinal cord. In the great majority of cases, these reflexes increase respiration and circulation. However, the two groups of reflexes are not exact opposites, because in cats in which powerful stimulation is applied to the pericardium [2-4], or to the mediastinal pleura [1], from which afferent fibers run to the medulla, or in which electrical stimulation is applied to the central ends of the aortic, sinocarotid, or vagus nerves [8,9], it ispossible to evoke not only depressor but also pressor reflexes.

In the present work, an attempt has been made to induce both pressor and depressor reactions by varying the strength of chemical stimulation applied to the interoceptors of the abdominal cavity.

METHOD

The experiments were carried out on acute preparations of cats under 1 g/kg intravenous urethane anesthesia. For the selective stimulation of the intestinal receptors we used the method described by V. N. Chernigovskii [6], which consisted of perfusing the organ while it retained only nervous connection with the rest of the body. We tried to isolate and perfuse the whole of the small intestine from the duodenum to the caecum, because although well-developed reflexes may be obtained initially from small sections of the intestine, the excitability of such a preparation becomes rapidly reduced, whereas if larger portions are used they remain excitable for long periods while perfused with Ringer-Locke solution. The chemical stimuli used were nicotine and acetylcholine, and were used in concentrations from $1 : 10^{-9}$ to $1 : 10^{-1}$ in Ringer-Locke solution.

The time at which 1 ml of the stimulus was injected into the perfusing system was recorded by means of an electromagnetically operated marker. Intervals of 10 minutes were left between injections, and these were increased to 30-40 minutes when concentrated solutions were used. The blood pressure in the left carotid artery was recorded using mercury and membrane manometers, respiration was measured using a Marie's capsule and a tracheal cannula, and the drop rate of the perfusate from the vein was also recorded. From the curves, changes

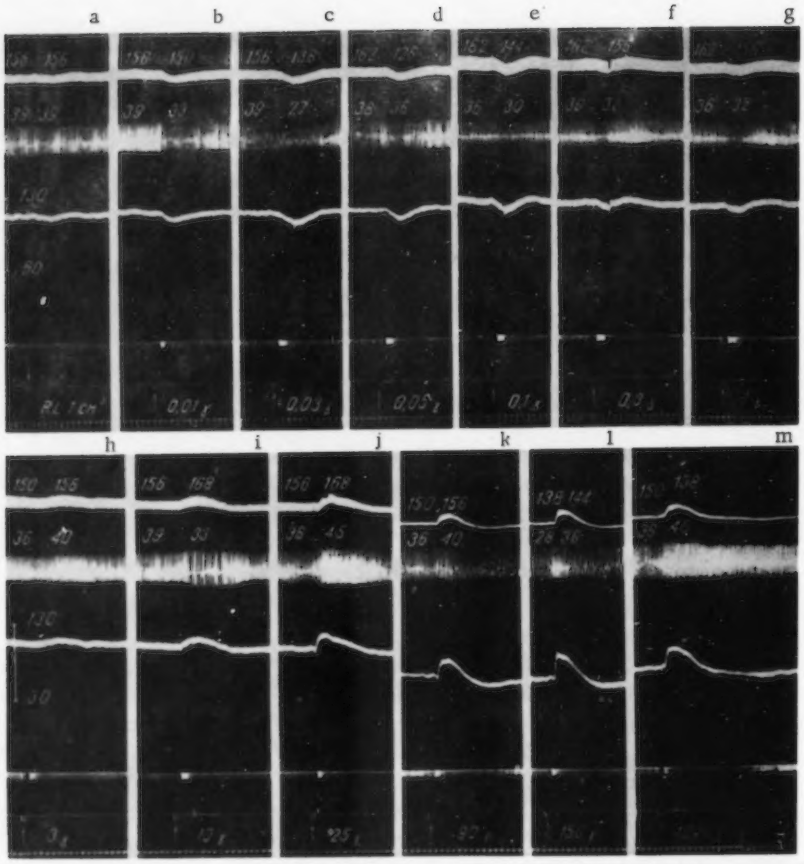

Fig. 1. Cardiovascular and respiratory responses to the injection of various concentrations of nicotine into the perfusate. Curves, from above downwards: arterial pressure (tonometer), respiration, arterial pressure (mercury manometer), drops of perfusate from vein, stimulus marker, time marker (5 seconds). Figures over the tonometer curve indicate number of heart beats per minute, numbers over the respiratory trace indicate rate of breathing (see explanation in text). ↓) injection of Ringer-Locke solution into the perfusate; ↑) injection of nicotine.

in the arterial blood pressure, heart beat, and respiration in response to any particular stimulus were read off and expressed as a percentage of the original level, and plotted as a graph; the percentage change in the quantity measured was plotted as ordinate, and the concentration of the stimulus as abscissa.

RESULTS

The cardiac and respiratory changes induced by nicotine and acetylcholine were closely similar.

Fig. 1 shows the traces obtained when the small intestine receptors were perfused with nicotine. The introduction of 1 ml of Ringer-Locke solution into the perfusate has practically no effect on any of the quantities recorded (Fig. 1, a).

Nicotine in doses from 0.01-1γ ($1 : 10^{-8} - 1 : 10^{-6}$) always caused a small but definite reduction in arterial pressure and a fall in heart and respiration rates (Fig. 1, b – g). Evidently, a "pure" depression developed only in response to 0.01 γ of nicotine (see Fig. 1, b) and the arterial pressure then returned gradually to its initial value. Large doses of the stimulus (see Fig. 1, c – e) first caused a greater fall in arterial pressure and a reduction in the heart beat; however, these reactions terminated abruptly as though under some counteracting influence.

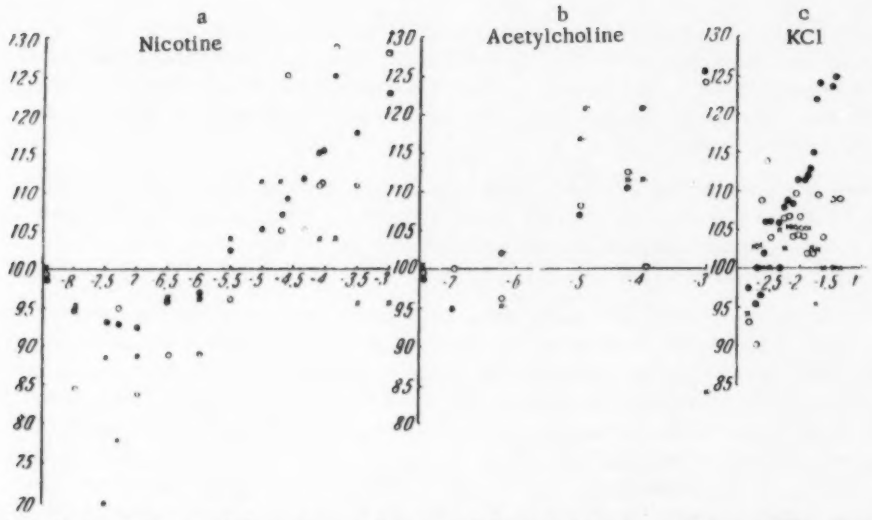

Fig. 2. Dependence of the nature and extent of the arterial pressure change (O), respiration (●) and heart rate (X) on the intensity of stimulation of the small intestine receptors by nicotine (a), acetylcholine (b), and potassium chloride (c).
Percentage change in the different quantities plotted as ordinate; minus one multiplied by negative logarithm of stimulus concentration plotted as abscissa.

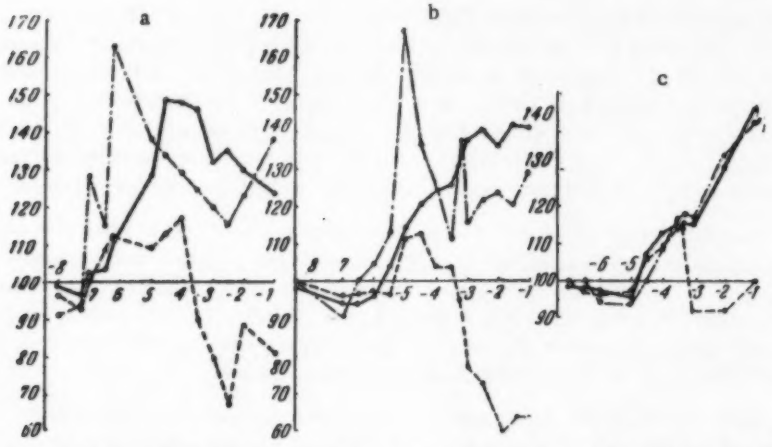

Fig. 3. Relationship between the nature and the intensity of the cardiovascular and respiratory responses to different intensities of small intestine receptor stimulation by acetylcholine, and the threshold at which transition from depressor to pressor response occurs. Symbols as in Fig. 2.
—)arterial pressure changes; – – –)changes in heart rate; – – –) respiratory changes.

That there was such an influence was confirmed by the fact that still greater doses of nicotine caused a biphasic reaction in which the small initial reduction in the arterial pressure rapidly changed over to a brief pressor response, and it was only after this had occurred that the pressure once more returned to the original value (Fig. 1, f, g). Evidently, 3 γ of nicotine is a threshold dose for the pressor reaction (see Fig. 1, h), although this same dose causes induction of a biphasic respiratory response. Even when 10 γ of nicotine are injected (see Fig. 1, i), when there was a marked increase in arterial pressure and heart rate, the respiratory change consisted of an increase in amplitude of the movements and a disturbance of the rate, which was usually reduced; only when the amount was increased to 20–25 γ was there a definite increase in respiration (Fig. 1, k).

The different quantities recorded reached maximum values at different stimulus strengths: the greatest increase in the heart rate was caused by 10-20 γ of nicotine, the greatest arterial pressure increase by 150 γ, and the maximum respiration was caused by 1000 γ. Higher concentrations caused a reduction in the response, so that when 300-1000 γ of nicotine were injected, the heart was slowed, the pressor response was reduced, but respiration was maximally stimulated.

These changes are shown in Fig. 2, a, which represents the results of one of the experiments (Fig. 1). On comparing graph a with the two others (b, c) shown in the same figure, it can be seen that the nature and the extent of the cardiovascular and respiratory responses are the same for the three different stimulators nicotine (a), acetylcholine (b), and potassium chloride (c) *.

The differences between the different graphs are purely quantitative: each substance shows a particular value for the threshold of the primary response and for the reversal of the reaction, and a range of values for which the reaction takes place in a particular direction; each substance also has its own particular value at which the reaction reaches a maximum, and a particular ratio of this value to the threshold dose. There is very little difference between the actions of acetylcholine and nicotine; there is some difference in the values for the changeover from depressor to pressor reaction and in the range of concentration over which depressor reactions and increasing pressor responses occur. Depressor effects were over a greater range and were more marked with nicotine, while acetylcholine produced greater pressor effects. The pressor response to nicotine occurred not only at a higher threshold, but also reached its maximum value more rapidly.

There are very clear differences between nicotine and acetylcholine on the one hand, and potassium chloride on the other. For the latter the threshold value is 1000 γ. Increasing the dose 10-14 times produces a maximum effect. All the changes described for nicotine and acetylcholine occur over this range.

Differences between the ways in which the respiratory response changes with concentration (Fig. 2) evidently does not depend on the properties of the particular stimulating agent. The graphs of Fig. 3 have been constructed from the results of three different experiments in which 1 ml of solution containing from 0.01 to 100,000 γ were added to the perfusate. Increasing the concentration showed that not only the respiratory response but also that of the circulation varied from one experiment to another. In some experiments, both reactions were maximal at a certain stimulus strength and began to fall with higher acetylcholine concentrations (Fig. 3, a). This effect occurred very frequently in the case of respiration (Fig. 2, c; Fig. 3, a, b) but sometimes the pressor response was reduced (Fig. 2, a; Fig. 3, a). As a rule, the maxima of the two responses do not coincide, so that in different experiments (with lower stimulus values) each reaction will reach its limit at a different concentration.

However, the respiratory reaction usually reaches its maximum earliest, even when it is depressed by small doses of the chemical stimulus (Fig. 2, a, b; Fig. 3, a, b), and also when higher stimulus concentrations are used (Fig. 2, c; Fig. 3, a, b). This effect is associated with the steep increase in the respiratory response between threshold and maximum, and in many cases with its lower threshold value. Even in cases when the threshold of one or another respiratory reaction was above that of the reversal of the pressor reaction (Fig. 2, a, b; Fig. 3, c), the two attained their maximum values at almost identical stimulus values.

It can be seen from Fig. 3 that the maximum pressor response depends upon its threshold value. When the threshold was low (Fig. 3, a), injection of 30 γ of acetylcholine caused a maximum increase in arterial pressure, and larger doses evoked a smaller reaction. When the threshold was higher (Fig. 3, b) the maximum reaction occurred at a dose of 3000 γ of the same substance, but in an experiment when the threshold pressor response was very high, a considerable increase occurred even with a stimulus dose of 100,000 γ.

The results indicate convincingly that the reflexogenous zone of the small intestine is not purely "pressor" and that stimulation of its chemoreceptors may produce depressor responses, either inhibition or stimulation of respiration, and either a fall or an increase in heart rate. In this respect it resembles the thoracic cavity. However, there are considerable differences in the changeover of the different responses to their opposites. With chemical stimulation of the pericardial receptors, weak stimulation brings about an increase in arterial pressure and a tachycardia, while stronger stimulation results in a fall in pressure and a bradycardia [2, 3]. Such a stimulation applied to the small intestine receptors causes changes in the reverse order (there is first a depressor and

* The results for the construction of graph "2, c" have been borrowed by permission of L. A. Baraz from her studies. — B. K.

then a pressor response, first a suppression and then a stimulation); the cardiac response is more complex (first an inhibition, then stimulation, then again inhibition).

The results presented do not solve the problem as to whether the opposite effects on circulation and respiration resulting from stimulation of the small intestine are due to different elements of the nervous system being involved, or whether they result from different levels of activity of similar elements of the reflex arc, as we attempted to show in the case of pericardial stimulation [2,3].

It must be realized that the different results obtained with identical stimulation of the interoceptors of the thoracic and abdominal cavities may be due to the fact that the afferent nerves of the former run chiefly to the medulla, and impulses from them induce a distinct set of cardiovascular and respiratory reflexes [6,7], whereas impulses from the latter run chiefly to the spinal cord and induce the functional changes we have recorded. A further complication is that in addition to the afferent spinal nerves, vagal branches also run to the intestine, and these contain afferent fibers of bulbar origin. Possibly it is this circumstance which accounts for the complex reversal of the cardiac response to chemical stimulation of the small intestine receptors.

SUMMARY

In experiments on acute cat preparations in which nicotine and acetylcholine were injected into the fluid perfusing the intestine, which retained only nervous connection with the body, the reactions induced depended only on the dose of the chemical substances. Low doses (0.01-1.0 γ) caused a slight drop in arterial pressure, and a fall in heart and respiration rates. On the other hand, large doses of 5-100 γ of these substances caused an increase in arterial blood pressure and a rise in pulse and respiration rates. The intensity of the reaction increased with increasing dose only up to a certain limit, which was not the same for the different responses; after the maximum has been reached, the blood pressure and respiration responses fall and the cardiac reaction becomes reversed, so that the heart rate falls in response to the action of nicotine upon the intestinal receptors.

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DEVELOPMENT AND SPREAD OF THE PULSE WAVE IN THE CRANIAL CAVITY

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The problem of the nature of the blood flow in the vascular system is of great importance in hemodynamics.

Great attention has been paid to the pulsation of the blood stream in the cerebral vessels. The results obtained by B. N. Klosovskii [3] and others [9, 10, 13] showed that the cerebral vessels did not pulsate when the skull remained hermetically sealed; the contrary claim has also been made [1, 2, 5, 16] that the blood flow in all the vessels, both arteries and veins, is pulsatile. However, much remains to be found out about the mechanism of transmission of the pulse waves within the cranial cavity.

We have shown previously in dogs [1], when studying the spread of the pulse wave from the heart to its entry into the sinuses, and following it as far as a more remote point in the superior longitudinal sinus, that despite the variation in distance from the heart, the time of spread of the pulse wave to these points was the same, having a value of 61-63 milliseconds.

Following up this result, we have studied the rate of spread of the pulse wave within the cranial cavity.

METHOD

The experiments were carried out on 23 adult dogs weighing 20-30 kg; morphine-urethane anesthesia was used, and heparin injected. The pulse rate was recorded using a piezomanometer, and cathode ray and string oscillographs (writing speed 60-250 mm/second).

In the first set of experiments the pulse rate was recorded simultaneously at the confluence of the sinuses and at the origin of the superior longitudinal sinus at a distance of 3-4 cm from the confluence. In the second set of experiments, recordings were made from the four points in the cerebral circulation as illustrated in Fig. 1.

RESULTS

If it is supposed that the pulse wave spreads in the vessels with a certain velocity in the direction of the blood flow, then from the direction of flow in the superior longitudinal sinus (toward the confluence of the sinuses) it would be expected that at the confluence the wave would develop somewhat later than at the origin of the superior longitudinal sinus. However, as can be seen from Fig. 2, the pulse wave in the superior longitudinal sinus and that at the confluence arise simultaneously.

In the second set of experiments, simultaneous recordings were made first of the pulse in the left and then of that in the right cerebral hemisphere, each being recorded together with the pulse in the superior longitudinal sinus; the pulse at the confluence was then recorded in turn simultaneously with that in the left and right hemispheres, and finally the pulse in both hemispheres was recorded (Fig. 3).

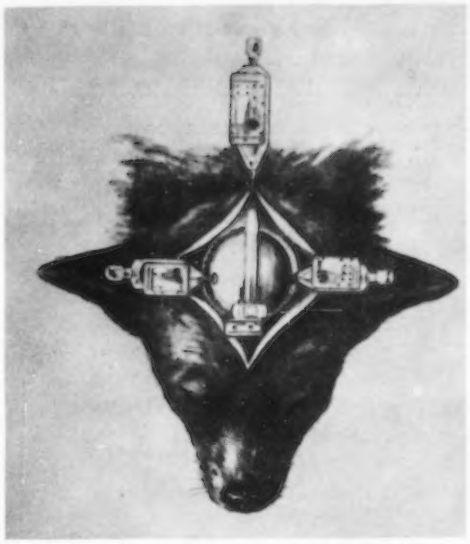

Fig. 1. Diagram showing the arrangement of the piezomanometers used for recording pulsations at 4 points (at the confluence of the sinuses, in the superior longitudinal sinus, and at two points in the cerebral hemispheres).

According to published results the cerebral pulse is derived from the pulsation of the cerebral arteries through the spread of the pulse wave along the blood stream, and if this is so it ought to occur somewhat before the sinus pulse. However, in our experiments there was no consistent time interval between the pulse waves at the points described. Thus, the time of spread of the pulse wave from the heart to the superior longitudinal sinus was 61 mseconds, from the heart to the left cerebral hemisphere 57 mseconds, to the right hemisphere 57 mseconds, and to the sinus confluence 63 mseconds. The differences in the above figures are within the limits of experimental error. Consequently, the pulse wave at all points in the blood stream develops practically instantaneously. Such a result is only possible if, due to the hermetically sealed skull, the increase in cerebral arterial pressure induced by cardiac systole is spread through the fluid medium and the incompressible mass of the brain in all directions equally and almost instantaneously; such a wave will travel with the velocity of sound in water, and will be compensated by transference of a sufficient amount of venous blood into the cerebral veins and of cerebrospinal fluid (CSF) into the spinal canal [2, 4, 5].

Thus, the CSF transmits the pulse wave from the arteries to the veins and cerebral sinuses. However, its hemodynamic function is not confined to a simple transmission of the pulse wave.

It has been known for a long time that the amplitude of the pulse wave becomes considerably reduced in the cranial cavity. Thus, the oscillations in intracranial pressure in the dog under morphine anesthesia are 4-5 mm of water [12]; the variation in intravenous pressure under the same conditions is 1-2 mm of mercury [16]. We have found that in a dog under morphine-urethane anesthesia, when the pulse wave is generalized, the pressure variation at the confluence of the sinuses is 20 mm of water inspiration, and 30 mm at expiration.

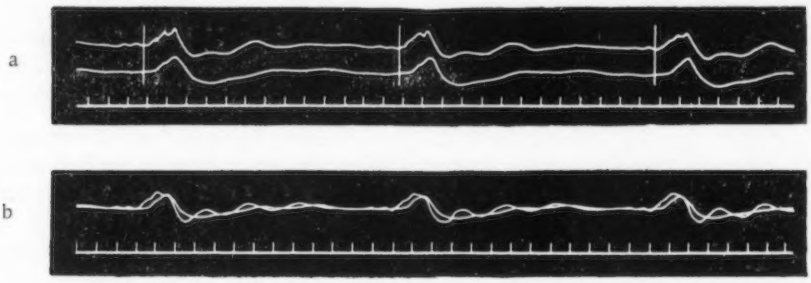

Fig. 2. Coincidence of pulse in the superior longitudinal sinus and at the confluence of the sinuses.
a) Upper curve – pulse at the confluence, lower curve – pulse in the superior longitudinal sinus; b) ditto with the traces superimposed. Writing speed 250 mm/second.

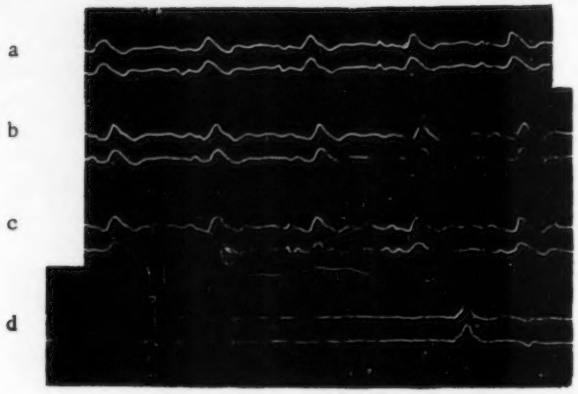

Fig. 3. Simultaneous occurrence of the pulse at 4 points in the blood stream. a) Upper curve — pulse in superior longitudinal sinus, lower curve — brain pulsation; b) upper curve — pulse at sinus confluence, lower curve — brain pulsation; c) upper curve — pulse at sinus confluence, lower curve — pulse in superior longitudinal sinus; d) upper curve — brain pulsation in right hemisphere, lower curve — brain pulsation in left hemisphere.

TABLE

Pressure in the Arteries of the Circle of Willis (in mm mercury)

Pressure in the internal carotid artery in neck	Pressure in brain	
	internal carotid artery	medial meningeal artery
114/78	104/72	—
74/40	84/64	—
96/72	—	92/66
96/60	—	96/56
94/56	—	90/54
—	—	130/82
		134/84
		130/80

It has been suggested [3, 9, 10, 13] that the pulsations of the blood cease on entering the skull so that the flow becomes steady on account of the skull being hermetically sealed, and because of the special structure of the cerebral arteries (the siphons of the internal carotid and vertebral arteries, the well-developed elastic layer in the wall of the cerebral arteries, the reticular arrangement of the arteries of the pia mater). However, direct measurements of the pressure in the cerebral arteries has shown that both the average and pulse pressure are reduced very little. Thus, according to Best and Taylor [7], in the large cerebral arteries the systolic pressure is 100 mm mercury, and the diastolic 65 mm. In measuring the pressure in all the arteries of the circle of Willis during operations [8, 14, 15], the following figures were obtained (see Table).

From these results it can be seen that the increase in pulse pressure in the cerebral arteries is of the order of several tens of millimeters of mercury, while the pulsatile variation in the CSF and sinuses is measured in millimeters of water. Thus, although the pulse pressure does not disappear, it becomes enormously reduced. However, this reduction does not occur principally in the cerebral arteries, where the pressure remains equal to that in the peripheral arteries of corresponding caliber, but in the transmission of the wave to the CSF, veins, and sinuses. Direct experimental evidence has been obtained which shows that when there is disturbance of the normal CSF circulation, the fluid becomes less able to act as a shock absorber and take up the pulse wave. Thus V. P. Osipov [6] found that hemorrhage through perforations in the brain was reduced by reducing the volume of CSF. Evidently, when the fluid is removed, the pulse pressure is increased, so causing further damage to the cerebral vessels. When CSF is withdrawn from the spinal canal, a considerable increase in the brain pulse is observed [11].

It appears probable, therefore, that the CSF is chiefly responsible for absorbing and transmitting the pulse wave; it accounts for the particular hemodynamic features of the brain: It maintains the high level of the arterial pressure necessary for the maintenance of an optimal blood flow, and greatly reduces the transmitted pulse pressure.

SUMMARY

Experiments were carried out on 23 anesthetized dogs, and heparin was injected. The pulse wave was recorded simultaneously at various points of the cerebral vascular system. It was shown that the pulse wave appeared at all points simultaneously, 57-63 milliseconds after the blood entered the aorta. This result confirms the idea that the cerebrospinal fluid plays a definite part in acting as a shock absorber and in transmitting the pulse wave through the cranial cavity.

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MUSCLE RECEPTOR EXCITABILITY IN RELATION TO CARDIAC ACTIVITY AND ARTERIAL PRESSURE

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A study of pressor reactions during muscular work due to alterations in the excitability of proprioceptors as induced by drugs, is of both theoretical and practical importance. M. F. Golovkina [2] has used this method in coldblooded animals. She decerebrated frogs by I. M. Sechenov's method, and observed the fall in blood pressure on cooling the gastrocnemius muscle or immersing it in a 0.5 novocaine solution. Changes in the cardiovascular system caused by cooling a muscle or by its treatment with novocaine may be due to the effective elimination of the proprioceptors. However, in order to obtain a complete proof, it is necessary to exclude the direct effect of cooling, which may involve the heart, and which by itself might produce a depressor effect [3, 10]. In working on coldblooded animals, we have therefore attempted to use the most delicate method of temporarily eliminating proprioceptors (depressing their excitability) by using anelectrotonus. Our starting point has been the fact established by us previously [5], that the excitability of muscle receptors may be either increased or decreased by being brought into contact with an anode or a cathode.

In warmblooded animals, which have a constant temperature and more highly developed thermoregulatory mechanisms than coldblooded species, proprioception was depressed by cooling the muscle or by the application of novocaine. At the same time, the general body temperature was controlled.

The object of the present investigation was to reduce the excitability of receptors in resting muscle by treatment with drugs, and to observe the effect on the cardiovascular system.

METHOD

The experiments were carried out on 60 autumn frogs (Rana temporaria) and on 20 adult rabbits. The first experiment was carried out on bulbospinal frogs in which the heart and gastrocnemius muscles were exposed. A silver choride needle electrode was inserted in one of the gastrocnemius muscles, and the indifferent electrode plate placed on the belly. One to one-and-a-half hours after making the preparation, the initial e.c.g. was recorded; the constant current was then applied and the effect of anelectrotonus or catelectrotonus on cardiac activity found. The current was then switched off, and the recording continued until the heart returned to its initial state. The current applied to the gastrocnemius ranged in strength from 3 to 30 ma.

The next experiment was carried out on acute preparations of unanesthetized rabbits. The effect on the arterial pressure of effectively eliminating the receptors of the triceps femoris was found. This effect was obtained by cooling the muscle in frozen physiological saline, or by applying a 2% novocaine solution; the latter was either applied to the surface of the muscle, or 2-3 ml were injected intramuscularly. Blood pressure was recorded from the common carotid by an aneriod or a mercury manometer. The rectal temperature was measured.

TABLE 1

Changes in Cardiac Activity Following "Unipolar" Application of an Anode or Cathode to the Gastrocnemius Muscle

Action on muscle	Change in cardiac activity					
	amplitude			frequency		
	increase	decrease	no change	increase	decrease	no change
Anode (120 tests)	–	55	65	–	59	61
Cathode (130 tests)	88	12	30	91	12	27

TABLE 2

Experiment No. 19 Without Anesthetic. Female Rabbit, weight 3.5 kg (16/II 1957)

Time at which exp. carried out (in hours and minutes)	Treatment of muscle	Site of action (paw)	Blood pressure (in mm of mercury)			Rabbit body temperature (in °C)
			before treatment	during treatment	after treatment	
14.00—14.05	Coil separation, 10 cm	Left	120	130	119	37.6
14.10—14.15	the same	Right	120	127	121	37.6
14.25—14.30	Cooling	Right	120	112	117	37.5
14.40—14.45	Coil separation 10 cm	Left	116	124	118	37.5
14.55—15.00	the same	Right	117	118	116	37.4
15.15—15.20	Coil separation 7 cm	Left	116	132	120	37.4
15.30—15.35	the same	Right	120	120	120	37.3
15.55—16.00	Novocaine injection	Left	121	115	115	37.3
16.15—16.20	Coil separation, 7 cm	Left	115	116	116	37.3

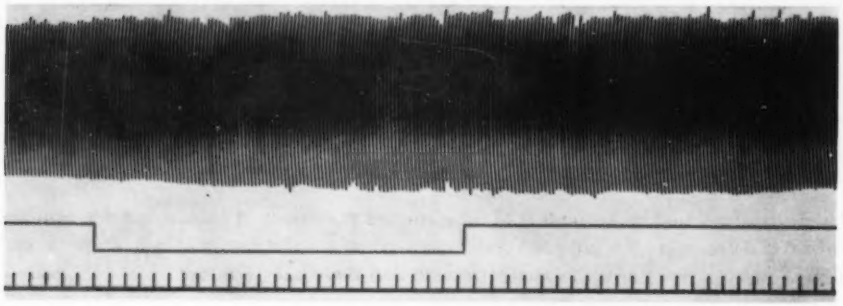

Fig. 1. Heart changes following application of a cathode to the gastrocnemius muscle. Curves, from above downwards: heart contractions, stimulation of gastrocnemius muscle, time marker (5 seconds)

RESULTS

Table 1 summarizes the results obtained on frogs.

We have seen that applying the cathode to a skeletal muscle caused an increase in amplitude and frequency of the heart beat in 88 out of 130 cases, and that only in an insignificant number of the tests was an inhibitory effect produced.

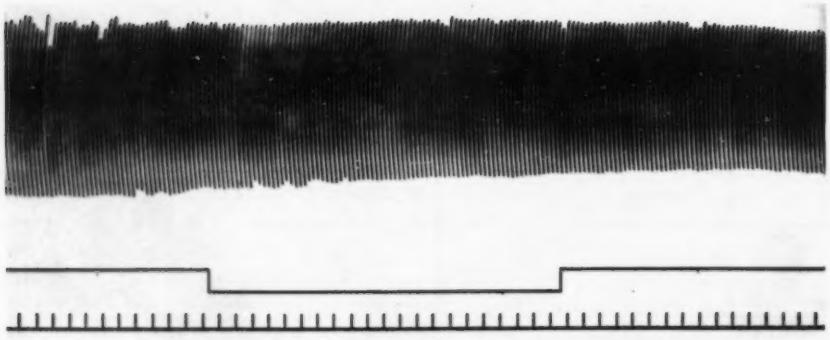

Fig. 2. Change in cardiac activity following treatment of the gastrocnemius muscle with an anode. Curves as in Fig. 1.

On the other hand, the effect of the anode almost always exerted an inhibitory influence, and a stimulating effect on the heart was never obtained.

Both direct observation and the e.c.g. records showed that the effect of applying the cathode to the gastrocnemius muscle was to stimulate the heart, the effect being greater on the amplitude of the contractions than on their rate (Fig. 1). The increase in the contraction amplitude was due to a stronger stystole and a greater relaxation during diastole. The depression in activity following treatment of the muscle with the anode was also due to changes in the amplitude of contraction (Fig. 2).

It must be noted that the changes described above were not very marked when a weak galvanic current was used; but evidently the mechanism plays an important part in regulating the cardiovascular system, particularly in the case of pathological proprioceptive disorders and central regulatory cardiac reflexes.

When the direct current was applied to the muscle, single contractions occurred, and the question naturally arises as to whether the cardiac changes resulted from these contractions. In some experiments, therefore, the current was established as follows: The switch was closed at a subthreshold value of the current, insufficient to induce muscular contraction; then gradually, by means of a rheostat, the strength of the current was increased to the required value. It was found that change in the performance of the heart was precisely the same whichever way the current was applied.

Thus, change in the excitability of resting skeletal muscle receptors induced electronically may have the same effect as muscular work [1, 5] in reflexly increasing cardiac output. There is reason to suppose that the stimulating effect caused by cathodic treatment is due to an <u>increase</u> in proprioceptive afferent impulses, while reduction in cardiac output following anodic treatment of the muscle results from a <u>fall</u> (elimination) of proprioceptor impulses.

This view has been confirmed by Edwards [11], who studied the effect of catelectrotonus and anelectrotonus on a single afferent axon innervating the muscle. According to him catelectrotonus caused an increase in the rate of impulses from a muscle spindle, while anelectrotonus had the opposite effect.

The next stage was to study the effect on the heart of temporary proprioceptor suppression in warmblooded animals. The experiments showed that even the temporary elimination of the receptors of a single muscle produces marked effects on the blood pressure. As an example, the results of a typical experiment on a rabbit are shown in Table 2.

As can be seen from Table 2, elimination of skeletal muscle reception by cooling or by novocaine injections causes a rapid fall in arterial pressure of 6-8 mm mercury on average, while stimulating the unesthetized muscle with current from an induction coil causes an increase in pressure. Current from an induction coil applied to a skeletal muscle which has been previously cooled or anesthetized with novocaine has practically no effect on the blood pressure, although the muscle itself contracts strongly. No appreciable body temperature change resulted from the cooling. Similar results were obtained in experiments on other rabbits.

Comparing the results of the experiments on frog and rabbit, it can be seen that different factors acting on resting skeletal muscle and altering its excitability cause reflex cardiovascular changes. The actions of anelectrotonus, novocaine, and cooling are similar. If the muscle receptors are temporarily eliminated by suppressing their excitability, reflex changes occur in the circulatory system and possibly also in other internal organs.

This leads to the question of in what way reduction or complete elimination of the muscle receptors affects the cardiovascular system. It has been suggested that the continuous flow of impulses from skeletal muscle receptors which maintain a certain tone but which are not actually contracted, has a continuous influence on the nervous centers and so regulates the circulatory system. In other words, the level of activity of the cardiovascular system, even at rest, appears to be maintained by a constant flow of impulses originating in skeletal muscle receptors which are at rest, but which have a tone. M. R. Mogendovich, A. K. Chuvaev, and G. Z. Chuvaeva have demonstrated a relationship between the tone of the arm muscles and arterial pressure, under clinical conditions [8].

The cardiovascular system reacts therefore not only to contraction or relaxation of skeletal muscle [4, 6, 8, 12], but also to an increase or decrease (elimination) of the excitability of the receptors of muscles which may be in a condition of comparative rest. These effects must be mediated by visceromotor reflexes [7].

SUMMARY

Experiments were performed on frogs and rabbits. A study was made of the dependence of the cardiovascular system on changes in proprioceptor excitability induced by anesthesia. It was shown that application of a cathode to a frog gastrocnemium causes an increase in the heart rate; on the other hand, application of an anode to the muscle depresses cardiac activity. Cooling the triceps femoris of a rabbit, or injecting novocaine into it, causes a rapid decrease in arterial pressure of 6-8 mm Hg. Consequently, not only muscular activity, but also change of excitability of the skeletal muscle proprioceptors is accompanied by reflex circulatory changes. These are visceromotor effects.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

HIGHER NERVOUS FUNCTION IN ADULT ANIMALS EXPOSED TO IONIZING RADIATION BEFORE BIRTH

COMMUNICATION II. IRRADIATION OF 18-DAY RAT EMBRYOS

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In several papers [1,2,3] we have described changes in the higher nervous activity of rats exposed to ionizing radiation (j-rays) during the intrauterine period, at the early period of organ formation, and at the 12th day of embryonic development. The embryonic period of the rat is 22-23 days. It has been shown that irradiating rats on the 16-18th day of pregnancy causes disturbances affecting the development in the young of the cerebral cortex, corpus callosum, Ammon's horn, and the corpus striatum [4]. A study of higher nervous function in animals irradiated at this period is of particular interest.

In the present paper we report results on changes in higher nervous function in adult rats exposed to radiation shortly before birth (on the 18th day of embryonic life).

METHOD

The day on which a female had copulated was determined from the presence of sperm in the vaginal secretion. (There was a possible error of 18 hours; the females were put among the males at 3 p.m., and were left there until 9 a.m. the following day). On the 18th day of pregnancy, the animals were exposed to 200 r of total x-ray irradiation, given at 20 r/minute. An RUM-3 apparatus was used, the tube voltage was 190 kv, current strength 15 ma, filters 0.5 mm cu and 1 mm Al, and focusing distance 40 cm.

The present paper is based on observations on 32 rats; of these, 16 were irradiated, and 16 others born at the same time but not exposed to radiation served as controls. The degree of spontaneous movement was measured in a special apparatus on the 30th and 40th days. When both groups were 45 days old, their higher nervous activity was measured by L. I. Kotlyarevskii's motorfood method. First a conditioned reflex to a 400 c/s tone attenuated 20 db was developed; then a negative conditioned reflex (differentiation) to a higher note of frequency 800 c/s attenuated 40 db was also established; after which a second positive conditioned reflex was established to the light of a 6 v red incandescent lamp. Afterwhich the stimuli were applied in the following sequence: lower note 5 times, light 3 times plus 1 negative conditioned stimulus (higher note) . The conditioned stimulus was presented for 10 seconds: 5 seconds by itself and 5 seconds together with the unconditioned food reinforcement.

After the animals had been thoroughly conditioned, tests were applied for the strength, lability and equilibration of the fundamental nervous processes; tests for the stability of the differentiation were made by a two-day fasting period, a caffein test was applied which included extinguishing and restoring a reflex, and a further test

TABLE 1

Comparison of Activity and Weights in Irradiated and Control Rats

Group of animals	Motor activity: no. of spontaneous movements in 10 m. on the 30th day	Motor activity: no. of spontaneous movements in 10 m. on the 40th day	Average weight of animal (in g) when 45 days old
Experimental	15.0±2.7	14.8±2.7	95.1±3.7
Control	6.0±1.0	5.3±1.0	120±2.7

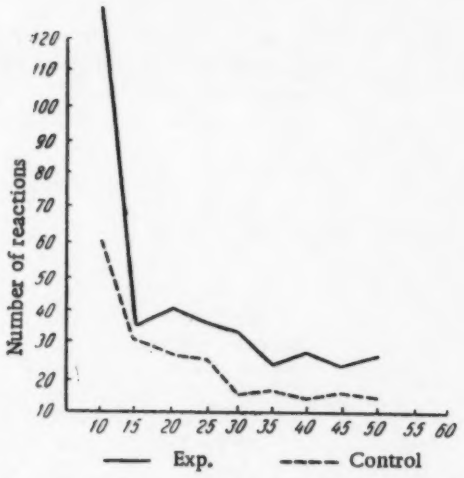

Fig. 1. Curves showing the extinction of the reaction in the intersignal period in the experimental group (——) and in the control group (-- -- --).

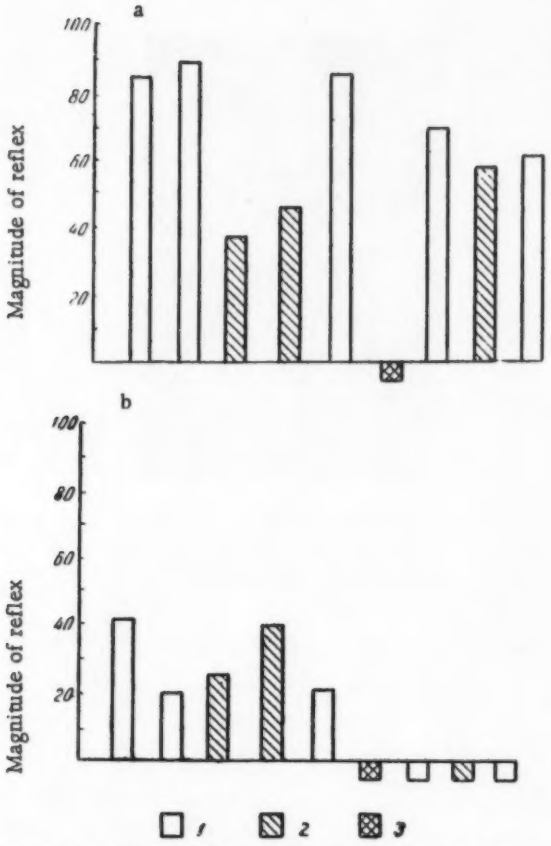

Fig. 2. Graphical representation of the results of experiments on (a) control and (b) experimental animals. In the experimental group there is a disturbance of strength relationships and an inhibitory after-effect. 1) sound (400 c/s); 2) light; 3) sound (800 c/s).

was made using external inhibition. Observations were made on the animals' behavior. The rate of development and the time required to stabilize positive and negative conditioned reflexes was determined. Average values of the conditioned reflex and of its latent period were calculated. Counts were also made of the number of movements between signals in the 10th, 15th, 20th, 25th, 30th, 35th, 40th, 45th, and 50th experiments. The results were treated statistically. Besides investigating the functional condition of the higher central nervous system, we also made morphological and neurohistological studies of the brains.

RESULTS

Spontaneous motor activity over a 10 minute period was measured in a special apparatus (an actograph) on the 30th and 40th day after birth in both experimental and control groups; the results showed a statistically significant increase in the number of spontaneous movements in the experimental group (Table 1). Probably the increase of more than twofold in the motor activity of the experimental group was due to a reduced inhibitory effect from the cortex and subcortical structures due to its delayed development. The reduced rate of growth in the experimental animals should also be noted (see Table 1).

A count of the number of reactions made between signals in experiments using conditioned reflexes showed that in most cases there was a statistically significant increase of them in the experimental animals. In Fig. 1 the curves show the changes in the number of these reactions (from the 10th to the 50th experiments) in both groups.

TABLE 2

Rapidity of the Development and Stabilization of a Positive Conditioned Reflex to Sound and to Light, and a Negative Conditioned Reflex to Sound

Kind of reflex	Animals			
	experimental		control	
	average number of combined applications			
	for devel. of cond. reflex	for stabil. of cond. reflex	for devel. of cond. reflex	for stabil. of cond. reflex
Positive conditioned reflex to sound	2.7±0.5	97.6±20.7	6.8±1	23±4.0
The same, to light	3.8±0.59	36.0±8.0	4.8±1	21.5±2·6
Negative conditioned reflex to sound	10.4±6	92.4±4.7	6.7±1.6	31.6±5·6

TABLE 3

Comparative Results of a Test on Extinction and Restoration of a Conditioned Reflex to Sound

Group of animals	occur. of extinct. ("0") "00000"	complete extinction	Restoration
Experimental	5.3±1.0	31.2±1.1	2.8±0.33
Control	7±1.0	13.2±1.0	2.6±0.21

Fig. 3. Brains of experimental and control 45-day old rats. Upper row) experimental group, lower row) controls.

The results show that when the experimental rats are placed in the compartment for test, at first a large number of reactions occur between signals, but as the experiments are repeated they become fewer; in the 30th experiment a constant value of 5-7 reactions per experiment is reached. The curve showing the reduction in the number of reactions in the experimental group falls more slowly and does not reach its steady state until the 35th experiment, when the value is 13 to 16 reactions per experiment, which is twice the value in controls.

The increase in the number of intersignal reactions in the experimental animals indicates a disturbed relationship between excitatory and inhibitory processes, with the excitatory process prevailing.

Rapidity of formation and stabilization of conditioned associations. In the experimental animals, the first appearance of a positive conditioned reflex to a sound occurred earlier than in the controls, but it took longer to stabilize and required a very large number of combined applications of the stimuli (about 100; Table 2). The differences in the second positive conditioned reflex are not statistically significant. In these animals it was particularly difficult to develop a negative conditioned reflex to sound (differentiation). Of the 16 control rats, only in one did we fail to stabilize the differentiation, while of the 13 experimental animals, failure occurred in 7. In the experimental group, the number of applications required to stabilize the reflex was three times greater than in the controls (significance at a level P=0.01).

Observations on the stereotype. Comparison of latent periods, magnitude of the conditioned reflex responses to positive signals, percentage of failures, and cases of failure of inhibition in the differentiations, showed that

in general the conditioned reflex activity of the antenatally irradiated animals was impaired. In them, there was as a rule some disturbance in the strength relationships, their condition fluctuated, often there was disinhibition of the differentiation, and there was rapid exhaustion in the second half of the experiment. Fig. 2 shows characteristic results for experiments on the two groups.

Once more, the special features in the experimental group must be pointed out: In some it was not possible to establish a stable stereotype, the temporary association remaining unstable during the whole of the observation period. Sometimes there was a dissociation between the artificial and the natural conditioned reflexes: The artificial reflex was present, but the natural one absent (the rat opens the door, but takes no food); in many cases the unconditioned reflex was also absent (bread was taken, but not eaten).

Functional tests. When an excessively large stimulus was applied (external inhibition), in 96% of the experimental animals there was a deterioration of conditioned reflex activity, which as a rule took the form of failure in one, two, or in all the subsequent positive conditioned reflexes to sound and light. Changes in conditioned reflex activity were found in 63% of the control animals, and frequently consisted only in an increased latent period and in a reduction in the size of the reflex after the application of external inhibition. A test in which differentiation was maintained for 3 minutes showed that of the experimental animals, not one was able to maintain the differentiation between stimuli, while in the control group, 30% passed this test. The average duration for maintaining a prolonged differentiation in the experimental group was 27 seconds, and in the controls 81 seconds.

A study of extinction and restoration of the conditioned reflex to sound gave the following results (Table 3).

From Table 3 it can be seen that in the experimental group, extinction occurs more slowly than in the controls. These results are statistically significant at a 0.01 probability level.

Tests with a two-day fasting period and administration of caffein showed invariably a weakness of the nervous processes in the experimental rats. After a two-day fast, the value of the conditioned reflex response to sound and to light was often reduced, and the latent period frequently increased (breakdown inhibition). In the control animals, these changes occurred comparatively rarely. After injecting caffein there was disinhibition of the differentiation, disturbance of the relative strengths of the responses, paradoxical and equalizing phases, and sometimes complete failure of the conditioned reflexes, or breakdown inhibition.

From the results on the speed of formation and stabilization of the conditioned reflexes, from observations on the stereotype, and from a study of the tests and rate of fall of the intersignal reactions (orienting reflexes), it may be concluded that the special features of the higher nervous activity of rats irradiated on the 18th day after conception are an innately lower working capacity of cortical cells, the failure being chiefly in the inhibitory process; there is a failure of nervous equilibration between the nervous processes with the excitatory process preponderating, a reduced mobility, and the development of inertia. It is important to stress the fact that with irradiation applied at this time, in many animals there was serious impairment of the power of the cortex to establish new connections: They werenot able to develop positive conditioned reflexes, and cannot assimilate a stereotype of stimuli.

Comparison of the histological studies and the results of the physiological tests was particularly interesting. Morphological and histological examinations made at the 7th, 14th, 21st, 45th, and 90th days (after birth) showed a definite developmental failure of the cortex in the experimental group.* At 45 days, at the start of the conditioned reflex study, the brain weight in the control group was 1.875 ± 0.067 g, while that of the experimental animals was 1.175 ± 0.053 g. The poor development of the brain, which affected principally the hemispheres, was very marked, and it could be seen with the naked eye that the occipital lobes did not cover the corpora quadrigemina (Fig. 3).

Post mortem histological examination showed that in all animals there were various degrees of failure of development of the upper cortical layers. They were very thin and contained few cells, particularly in the dorsomedial parts of the hemispheres; Ammon's horn was poorly developed, and the corpus callosum was either reduced or absent. The nerve cells of the cortex were also changed, being vacuolized, crenated, and partially lysed.

*Experiment performed by I. Artyukhina

It has therefore been demonstrated that embryos irradiated with 200 r on the 18th day develop into adults which show grossly abnormal structure in the hemispheres and hippocampus. Evidently, the changes observed in the corpus callosum are secondary and follow from the smaller number of neurones which in turn results in there being fewer commissural fibers.

The defects in higher nervous function revealed in our experiment may be ascribed to the failure of the upper layers of the cortex to develop [2, 3, 4]; the behavioral effects observed included a reduction in the strength of the nervous processes, a failure of the cortex to form new connections, and a marked reduction in the strength of the inhibitory process. It appeared that the reason for the reduction in conditioned reflex activity, which was most marked in the case of sound stimulation, was the difference in the strength of the stimuli. With stronger sound stimulation, the functional cortical failure was better shown, and breakdown inhibition occurred. A further important factor in the disturbance of the higher nervous functions was the low degree of development of the hippocampus; recent electrophysiological investigations have shown that the hippocampus plays an important part in the formation of temporary connections by inhibiting the orienting reflex. It may be that the slow decay of the reactions occurring between signals, which hindered the stabilization of the conditioned reflex, results directly from the innately low level of hippocampal development in the animals used in our experiments.

SUMMARY

Female white rats were irradiated with a single total x-ray irradiation of 200 r on the 18th day of gestation. A congenital reduction in the working ability of the cortical cells was demonstrated by behavioral studies of higher nervous function in the adults. Apart from the weakening of the excitatory process, inhibitory power was especially diminished, while in a number of rats the ability of the cortex to form new connections was markedly impaired; there was also a disturbance of the equilibration between the different nervous processes, the excitatory process prevailing, and there was a considerable inertia. The upper cortical layers, the hippocampus, and corpus callosum, were all under-developed.

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••In Russian

ADRENALIN-LIKE SUBSTANCES AND THE BIOLOGICAL ACTIVITY OF THE BLOOD IN DIENCEPHALIC DISORDERS

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The pathology of the diencephalon, and particularly of its hypothalamic area, which contains the so-called autonomic centers, has been extensively investigated in the Clinic for Nervous Diseases of the I-Moscow Medical Institute. The appearance at an early stage in a disease of clinical signs of diencephalic involvement is of great practical importance; frequently these disturbances constitute the principal symptoms and may determine the subsequent course of the disease.

Neurophysiological and biochemical methods make it possible to reveal small changes due to disturbance of the subcortical autonomic centers which would not be revealed by the usual clinical tests. A detailed clinical and physiological examination may indicate diencephalic disorder in organic cerebral disease, and in various infectious or toxic conditions, and in disturbances of either temporary or permanent disorders of the relationship between cortex and subcortical structures.

We have previously shown that a single determination of certain physiological or biochemical constants is not sufficient to reveal the condition of the fundamental physiological systems. We have applied various tests and loads [1, 2, 3] which have enabled the limits of homeostasis to be determined, and the adaptive reactions to be measured; this process facilitates diagnosis, and distinguishes the primary pathological condition from its secondary manifestations. In this connection a study of neurohumoral regulation is of great importance as an index of the condition of the sympathetic and parasympathetic divisions of the autonomic system.

For many years we have studied patients with various abnormalities affecting the adrenalin-like substances (ALS), acetylcholine concentration, or sympathetic and parasympathetic substances of the bloodstream [4, 5, 6].

In the present paper we present the results obtained during brief spells of cooling or heating the body designed to induce a condition of "stress" (the term used by Selye).

METHOD

The ALS concentration in the blood was determined by Shaw's method, as modified by A. M. Utevskii and M. G. But [8] for the detection of dehydroforms (reoxidized forms of ALS) in the presence of ascorbic acid. A detailed description of the method has been given previously [4, 5, 6]. The fraction of unoxidized adrenalin in the complex was described by the coefficient of specificity (KSp). We have somewhat arbitrarily assumed that when the KSp is equal to or greater than 2, the ALS fraction contains only adrenalin. When the KSp is less than 1, no adrenalin is present, and the whole complex consists of unidentified ALS, which have been described by A. M. Utevskii by the term "chromogens". If the KSp lies between 1 and 2, the substrate investigated contains both adrenalin and chromogens. Their ratio is indicated by the value of the KSp. We also calculated the total ALS (total adrenalin) and the ratio of the dehydroforms to total adrenalin (dehydroadrenalin coefficient).

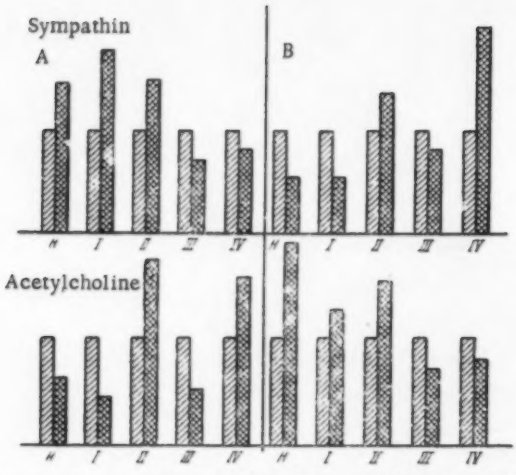

Fig. 1. Diagram showing the effect of (A) cold and (B) hot tests on the sympathin and acetylcholine content of the blood in healthy subjects (H) and in patients with diencephalic disorders (I-IV: 4 types of reaction) (diagonal shading – before testing, cross shading – after test)

The biological activity of the blood (or blood serum) was determined by applying it to the isolated frog heart or to leech muscle. We used the method developed at the Institute of Physiology of the USSR Academy [5, 7] which is based on observing changes in the amplitude and frequency of the contractions.

Tests were also made on 22 healthy human subjects, and on 112 patients with various diencephalic disorders; they were tested on an empty stomach, and immediately after a period of immersing the hands up to the wrist in cold water at 4° (the cold test), and immediately after a three-minute period with the hands immersed to the wrist in hot water at 44° (the hot test).

In considering the results obtained, it must be remembered that: 1) Increase in sympathetic activity of the blood may be due to an increase in concentration of sympathomimetic substances (sympathins) as well as to a reduced concentration of parasympathomimetic substances (parasympathins), particularly acetylcholine; 2) sympathetic activity of the blood may be reduced either by a reduction in the concentration of sympathins, or by an increase in parasympathins, particularly acetylcholine.

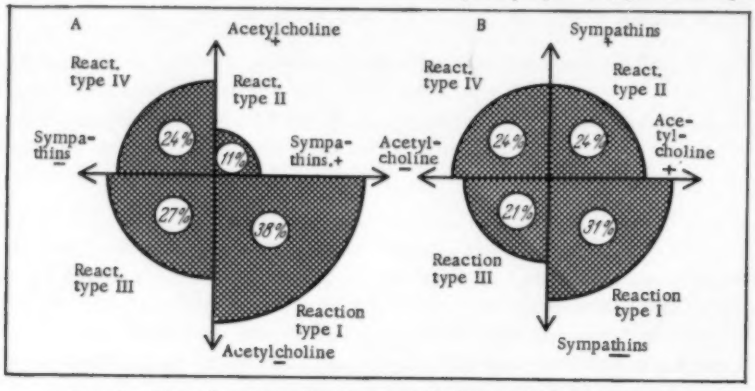

Fig. 2. Classification of patients with diencephalic disorders into 4 groups; reactions to (A) cold and (B) hot tests according to degree of increase (+) or decrease (-) in the sympathin and acetylcholine blood levels.

As we have shown previously [4, 5, 6], increase in sympathetic activity may depend firstly on an increase in the KSp, i.e., on the presence of the most active unoxidized forms of adrenalin, and secondly on an increase in the total ALS in the form of chromogens (noradrenalin ?). Similarly, a reduction in sympathetic activity may be caused either by a reduction in KSp, i.e., by a disappearance or a reduction in the amount of unoxidized forms of adrenalin, or by a reduction in the total ALS through fall in the content of chromogens (noradrenalin ?).

Because blood always contains different amounts os sympathins and parasympathins, its action on the isolated frog heart was tested repeatedly before and after adding atropine. The difference in the effect produced enables the true sympathin level of the blood to be determined.

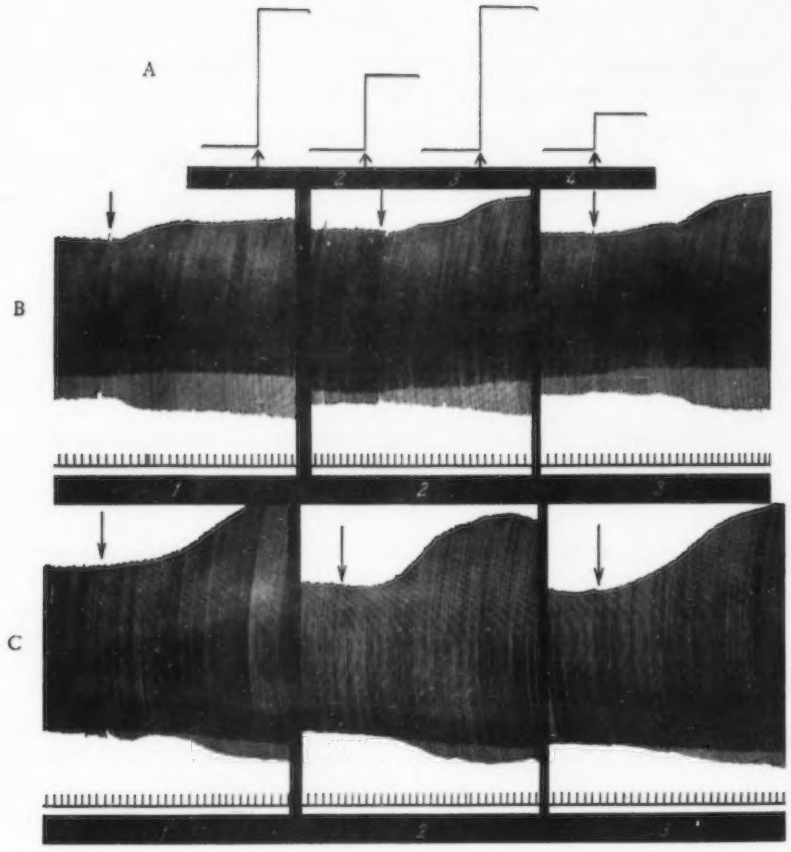

Fig. 3. Biological activity of the blood as shown by heat test (reaction type IV). A) Acetylcholine level in blood (1, 3 — effect of standard solution of acetylcholine [1 : 10^{-5}]; 2) level of acetylcholine before test; 4) level of acetylcholine after tests); B) effect of patient's serum, taken before test, on the isolated frog heart; C) effect of serum from patient taken after test on isolated frog heart; (B and C: 1) dilution 1 : 1500; 2) dilution 1 : 150; 3) dilution 1 : 15).

RESULTS

In healthy human subjects, the cold test causes a marked sympathetic effect on the frog heart (the positive inotropic effect of the plasma on the heart increases by 40-50%) while the acetylcholine level is reduced, on average by 40%. In some cases, after the cold test there is a reduction in the concentration of sympathins, and at the same time a marked fall in the amount of acetylcholine. Thus the relation of the sympathins to the parasympathins alters so as to result in an increased sympathetic activity.

In 65% of the cases, the ALS content increased, but the KSp either remained unchanged or was reduced. Some increase in the level of dehydroforms (reoxidized forms) of ALS was also found. It may be concluded, therefore, that in healthy subjects the cold test causes an increased sympathetic activity of the blood, chiefly on account of an increase in the chromogen (noradrenalin?) fraction, and a reduction in acetylcholine.

In healthy subjects the hot test produces a well marked parasympathetic effect (the positive inotropic effect of the plasma on the frog heart is reduced or fails entirely), and the acetylcholine level increases on average by 175%. There are no characteristic changes in the ALS, KSp, or dehydroform ALS; their values increase, decrease, or remain unchanged in approximately the same numbers. However, as a rule the variation was small and had no effect on the sympathetic activity of the blood, which in most cases was reduced by the hot test.

TABLE

Adrenalin-like Substances and the Biological Activity of the Blood in Patient A. (Diencephalic Disorder Affecting Endocrine Balance)

Test	ALS (in γ%)	DALS (in γ%)	TA (in γ%)	KSp	DAC	Inotropic effect in dilution 1:1500	Acetylcholine (in γ%)	Group assigned as result of tests
Cold	8.8	0	8.8	0.8	0	197	0.92	IV
	8.7	0	8.7	0.7	0	78	1.48	
Heat	6.7	0	6.7	0.4	0	129	0.94	IV
	7.8	1.2	9.0	0.8	0.15	184	0.36	
After course of x-ray therapy (400 r applied to diencephalic region)								
Cold	11.3	0	11.3	0.9	0	38	0.62	I
	12.2	0	12.2	0.8	0	113	0.24	

Note. 1. ALS) adrenalin-like substances, DALS) dehydro-adrenalin-like substances, TA) total adrenalin, KSp) coefficient of specificity, DAC) dehydro-adrenalin coefficient. 2. Line one – before test, line two – after test. 3. The graph shows a 7% increase in the positive inotropic effect as compared with that due to a standard solution of adrenalin diluted $1 : 10^{-7}$.

Different diencephalic disorders may cause the most varied changes in ALS content and in the biological activity of the blood, whose limits are determined by the process of homeostasis. In each separate case, they may be caused by a preponderance either of sympathetic or of parasympathetic tone, by the time course of the reactions, through a replacement of the primary by secondary pathological symptoms, and by the lability or inertia of the nervous processes.

From the results of the cold and hot tests, patients with diencephalic disorders may be referred to one of the following groups (see also Figs. 1 and 2).

Cold test. First group – increased sympathetic activity and reduced acetylcholine. Qualitatively the results were the same as in the control group. In diencephalic disorders, considerable changes in the amount of the substances investigated occur, and are often several times greater than the normal physiological variation. The KSp often has a much higher value than in contols, thus indicating the presence of unoxidized forms of adrenalin.

Group II – increase in both sympathetic activity and acetylcholine level. N. I. Grashchenkov and G. N. Kassil' [1] have suggested that the primary sympathetic reaction is rapidly compensated by an increased parasympathetic response which may even overcompensate, and the acetylcholine level may increase 3-4 times.

Group III – simultaneous reduction in sympathetic activity and acetylcholine level. The ALS are reduced; the KSp either falls or remains unchanged. The sympathoadrenal apparatus is less reactive, and there is a compensatory reduction in parasympathetic tone.

Group IV – reduced sympthetic activity and increased acetylcholine. The ALS content falls, the KSp falls or remains unchanged. This reversed cold test result is frequently observed in cases of severe disturbance of the regulatory and compensatory mechanisms.

The results obtained show that diencephalic patients of all four groups show differences which are significantly greater than the fluctuations in healthy subjects.

Hot test. Group I – increase in acetylcholine and decreased sympathetic activity of blood. Qualitatively the tests were not different from normal; however, the acetylcholine level increases on average 33% less than in the control group. This indicates a reduced parasympathetic nervous response.

Group II – simultaneous increase in acetylcholine and in sympathetic activity. In a considerable number of cases there was an increase in ALS. Probably the primary parasympathetic reaction was rapidly compensated by increased sympathetic activity. The KSp was more frequently increased than in normal subjects, indicating the presence of unoxidized forms of adrenalin.

Group III – simultaneous fall in acetylcholine and sympathetic activity. In some cases the ALS level was reduced, as was also the KSp. This result indicates parasympathetic failure compensated by a relative fall in sympathetic tone.

Group IV – the acetylcholine was reduced by 34% on average, and the sympathetic activity was increased up to 5-6 times (Fig. 3). In this group the ALS was usually increased and the KSp raised. These results were usually obtained in cases of severe disorders of the regulatory and compensatory mechanisms.

In healthy subjects, when the hot test was applied, the ALS sometimes increased without there being any increase in sympathetic activity of the blood, because of the considerable increase in the amount of acetylcholine. Evidently, increase in ALS in the blood in the hot test is associated with the "stress" nature of the test.

In diencephalic patients, there is more frequently an increase in ALS and a raised KSp than in healthy subjects. In these patients there is also an increased acetylcholine level, both in the heat and cold tests, which is compensated by an increased ALS due chiefly to unoxidized forms of adrenalin (the KSp is raised).

Patients on which both the hot and cold tests were applied were very interesting. As a rule the results coincided, and both indicated that the patient should be referred to a particular group. Application of various therapeutic measures (x-ray therapy, nasal therapy, or hormone therapy) in many cases enabled the normal autonomic balance of the body to be restored (see Table).

Thus in routine clinical examination a single (hot or cold) test is sufficient. We carried out both tests only when there was difficulty in distinguishing the primary pathological signs from secondary compensatory processes.

From the results obtained we have drawn the following conclusion:

From studies of changes in the biological activity of the blood and of its content of adrenalin-like substances, it is possible to obtain evidence as to the condition of the autonomic nervous system and of its adaptive (homeostatic) mechanisms in healthy subjects and in patients with diencephalic disorders.

In healthy subjects the cold test causes an increase in sympathetic activity and a fall in the acetylcholine level of the blood. The hot test increases acetylcholine and decreases sympathetic activity.

The change in the biological activity of the blood depends on the content both of adrenalin-like substances and of acetylcholine.

In different forms of diencephalic disorder, the cold and hot tests cause characteristically different changes in the amount of sympathetic and of parasympathetic substances. We have found four different reactions corresponding to different regulatory mechanisms and different limits of homeostasis in various diencephalic disorders.

SUMMARY

Measurements have been made of the physicochemical and biological properties of the blood, based on tests designed to find the subjects' reactions to stimulation. From these it was possible to determine the limits of homeostasis and the nature of the adaptive reactions, and the results were used diagnostically; it was also possible to distinguish between primary pathological processes and secondary compensatory phenomena. Measurements were made of the concentration of adrenalin-like substances, acetylcholine, sympathins, and parasympathins in normal subjects and in those with diencephalic disorders. The two tests were based on the reaction to heat and to cold. Four types of disturbance of control and compensatory mechanisms were found.

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THE DOG ECG AFTER LIGATURE OF THE CORONARY ARTERY (EFFECT OF POSITION OF THE FORELIMBS)

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We have shown previously [6] that in healthy dogs the electrocardiogram (ECG) depends on the position of the forelimbs. Despite the published claims of the variability of the canine ECG, we have shown that for a given position of the forefeet, the shape of the ECG is constant.

The object of the present investigation has been to determine whether the position of the front legs affects the ECG when some pathological condition such as a myocardial infarct is present, and also to study the ECG in relation to different limb positions with the animal lying on its side.

METHOD

Experiments were made on 11 mongrel dogs of various weights and ages. A double anesthetic was used. Evipan was injected intravenously, and the operation continued under oxygen-ether. The thoracic cavity was opened at the fourth left intercostal space. A ligature was then placed on the descending branch of the left coronary artery at its origin. The pericardium was opened and the vessel tied without procaine being injected into the pericardial cavity, and without subepicardial infiltration of the vessel.

The ECG was recorded on a triplex apparatus with the animal lying on its right side. Records were made before the operation, after giving the anesthetic, after opening the thoracic and pericardial cavities, after dissecting out and after ligaturing the descending branch of the left coronary artery, and during and after the tying off of this artery. ECG records were made 2, 5, 15, 30, and 60 minutes and 2, 3, 4, and 5 hours after the operation, as well as daily for several days afterwards. The greatest time for which records were made was 220 days after the ligature. During the recording the front legs were kept in a fixed position, and the effect of varying this position noted. Initially the legs were placed in position A, with one leg over the other in the midline. Recordings were made of the effect of simultaneously moving the right leg forward and the left backwards (position B) and of reversing their relative position (position C).

RESULTS

It has been previously shown in healthy dogs that these positions bring out maximum ECG changes.

In some dogs, for 2 or 3 days after the operation it was not possible to follow the ECG changes, because the typical picture of myocardial infarct was obscured by ventricular extrasystoles.

We have shown that the position of the front limbs has a definite effect on the ECG both immediately after ligature of the coronary artery, and for the succeeding days and weeks (Fig. 1). Usually marked, though individual differences appear, the Q and T waves are altered, as is also the ST interval. In the B position the ST_1 interval

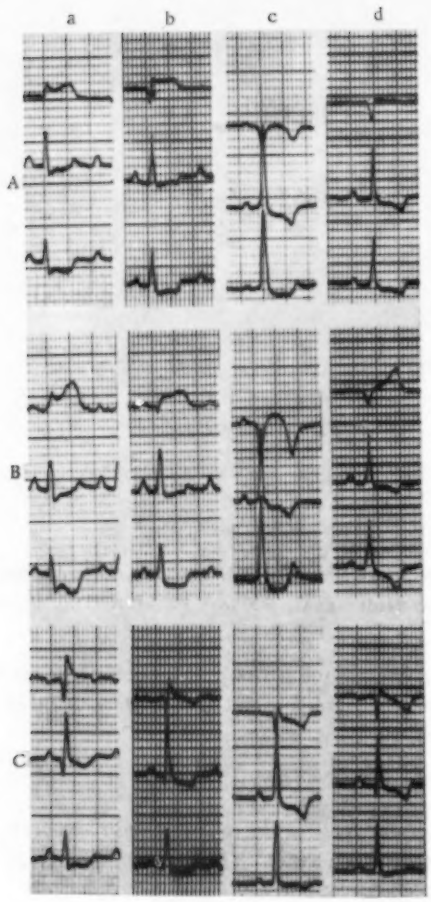

Fig. 1. Effect of changes in limb position on the ECG before and after ligature of the descending branch of the left coronary artery; standard leads.
A) Midway position with the front limbs together; B) right limb forward, left back; C) left limb forward, right back; a) position of the limb after 30 minutes; b) after 2 hours; c) after 2 days; d) 7 days after ligaturing the descending branch of the left coronary artery.

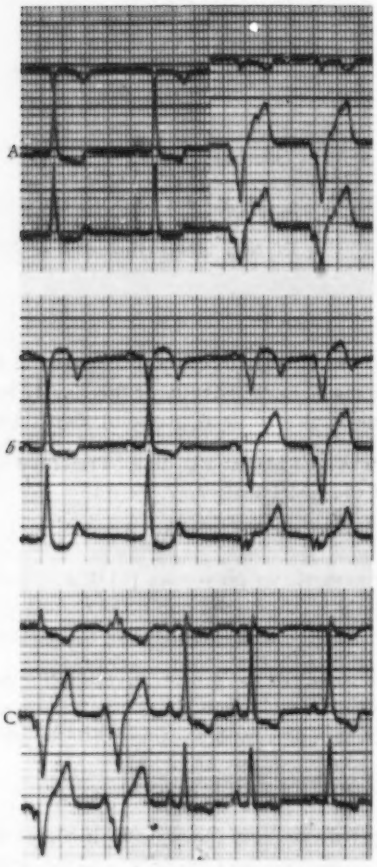

Fig. 2. Effect of changes in the position of the front limbs on the shape of the ventricular extrasystoles. Curves as in Fig. 1.

is displaced upwards, and the ST_2 and ST_3 intervals downwards. The ECG is the same as that found for myocardial infarct of the anterior wall in man. In position C there is practically no displacement of the ST interval. Only occasionally, in the first 2-3 hours after the ligature, is there a displacement of the ST_2 and ST_3 intervals downwards, and exceptionally there may be a small rise in the ST_1 interval.

Changes in the Q and T waves, which are found chiefly in Lead I and in positions B and C, are different, and sometimes occur in opposite directions. With the legs in the B position, the QS wave is deep and the negative T wave has a large amplitude.

The position of the front limbs also changes the shape of the ventricular extrasystoles. Fig. 2 shows results obtained on the second day after the ligature, when the sinus rhythm was almost completely obscured by these extrasystoles.

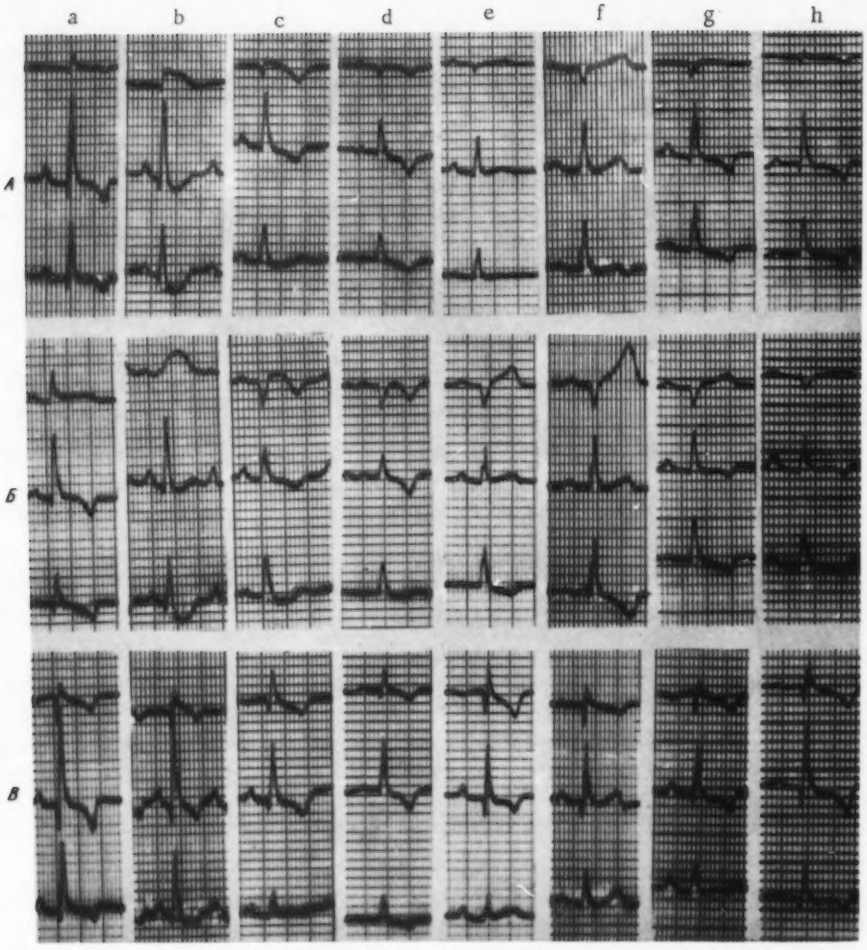

Fig. 3. Changes in the ECG following ligature of the descending branch of the left coronary artery in dogs.
a) Initial ECG; b) after 30 minutes, c) on 1st day; d) on 2nd day; e) on 9th day; f) on 26th day; g) on 36th day; h) on 56th day after ligature. A,B,C) as in Fig. 1.

Changes in the ECG following the ligature are shown in Fig. 3. The greatest change is shown for the position B. Thirty minutes after the ligature it can be seen that the ST_1 portion lies well above the line, that is to say it begins immediately at the peak of the reduced R wave and merges with the increased T wave to form a high arc. The ST_2 and ST_3 intervals are displaced below the base line. After 24 hours, ST_1 has fallen and at the same time a negative symmetrical T_1 wave with a sharp summit has formed (coronary type T wave). The T_3 peak has become positive. The ST_1 interval forms an arc directed upwards, and even by the second day after the ligature has returned to the baseline. Simultaneously with the changes occurring in the ST interval and the T wave, after 24 hours there is a deep and broad QS_1 wave. Marked changes occur by the 9th day: There is a high positive T_1 wave, T_3 has again become negative. By the 26th day T_1 has become higher (8mm), after which it gradually becomes smaller, and the ECG stabilizes with a broad QS_1 wave which may be observed for 220 days after the ligature.

In position A, the ECG changes resembled those described, but were less well shown, and by the 56th day were scarcely different from normal.

In position C the only change was in the size of T_2 and T_3, and even then the changes occurred only quite some time after the ligature was made.

Similar changes were found in the remaining 8 dogs, but they occurred at different times. In them the ST interval was well shown, and in position B was present 2 minutes after the ligature had been established. At this stage the T_1 spike was higher than the R_1. The coronary T notch was formed by the 3-7th hour after ligaturing. The high positive T wave appeared by the 4-11th day after tying the ligature, and reached its maximum value later; then the ECG began to return to normal (by the 17th day after the ligature, no change remained).

Records obtained in position C, apart from two cases where there was a small displacement downwards of the ST interval, showed no change. In a few dogs, immediately after the ligature there was a displacement of the ST_2 and ST_3 intervals downwards, and there was sometimes a change in the value of the T_2 and T_3 portions.

ECG changes recorded in position A after ligature could be followed only when the initial ECG in this position was approximately the same as that in position B. The greater the resemblance between the curves in the A and B positions, the better were the changes in position A shown.

In most of the experimental group, comparatively little change in the ECG was caused by the anesthetic, opening of the thorax and pericardium, dissecting out the coronary artery, or by the actual tying of the ligature.

During the anesthetic and after the ligature, no respiratory arrhythmia showed on the ECG. This effect was ascribed to diffuse myocardial damage [5] and is not to be attributed to blocking the termination of the vagus nerve. In 2 dogs the respiratory arrhythmia did not recur until the 86th day after ligaturing the coronary artery.

Our results throw doubt on the validity of interpretations of ECGs obtained with myocardial infarcts where there has been no control over the position of the front legs. We will now compare briefly our results (chiefly those obtained in position B) with various other investigations into ECG changes following coronary ligature.

V. G. Zhil'tsov [2] made a study which was continued for 10 months after such a ligature had been made. In the initial ECG, T_1 and T_2 waves were present; Q_1 was absent, but Q_2 and Q_3 present; T_3 was level with the baseline; the $ST_{1, 2, 3}$ intervals lay on the baseline. Six hours after ligature, ST_2 and $_3$ were displaced downwards by more than 2 mm. By the 21st day, a deep Q_1, Q_2 wave developed, and there was a negative T_1, $_2$, and $_3$. An ECG taken 9 months later showed a more negative T_1 wave, which was reduced in Lead III. Comparison of these results with the ECG changes obtained in our experiments suggests that the author's first two ECGs were recorded in positions A or B, and subsequently in position C, which would explain the great differences between the results obtained.

Results obtained by B. A. Lapin [4] may be explained in a similar way; immediately after the ligature, he found changes typical of myocardial infarct, but later records did not differ from normal.

The results of our experiments do not agree with those of L. G. Kopteva and her coworkers [3]; the first two changes they recorded after ligature of the coronary artery showed a marked Q_1 wave and a negative T_1. In our experiments the first change affected the ST interval, and at the same time there was an increase in T_1, and only later did the deep Q and negative T_1 appear.

Although in some of our experiments there was some change in the ECG during the operation, it did not appear that any such change was appreciable before the ligature was tied, as has been claimed by G. N. Aronov [1] (appearance of coronary T wave, displacement of ST interval downwards). In our view, these changes occur because the operation causes an alteration in the position of the heart. In our experiments we never found a single case of an ECG being changed by the coronary artery being dissected out, or any differences between the traces recorded before, during, and after coronary ligature. Immediately after the ligature had been tied, isolated or grouped ventricular extrasystoles occurred.

The observations described agree with those of Wegria and his coworkers [8], who found that after completely freeing the coronary artery, marked changes in the ECG always occurred; this was confirmed by Lengyel [7], who found that after ligaturing there was always an increased T wave, which only later became negative. However, unlike us, both authors found an initial change in the T wave and only later in the ST interval. In our experiments, both changes occurred at the same time, or else the shift of the ST interval occurred before the change in the T wave.

Our results indicate that position B is the best for studying the change in the ECG following ligature of the descending branch of the left coronary artery. Changes in the ST interval probably constitute the principal sign of myocardial damage, because they were not found in healthy dogs, while the Q and T waves may vary in normal animals according to the position of the front legs.

SUMMARY

Changes in the ECG were studied on 11 dogs after ligating the descending branch of the left coronary artery.

Changes in the position of the front limbs altered the Q and T waves, and had a considerable effect on the ST interval. It is therefore of importance to maintain the front legs in a fixed position when recording the electrocardiogram.

Changes in the ECG after myocardial infarction are most marked when the dog lies on the right side with the right front leg forward and the left back.

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CHANGES IN ELECTRICAL EXCITABILITY AND MUSCLE RESPONSE SPEED IN DOGS WITH EXPERIMENTALLY INDUCED TUBERCULOSIS

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In our previous researches we have shown that in dogs with tuberculosis the chronaxy increases as the infection progresses, and attains a maximal value in the final stage of the disease. When the course of the disease is arrested, and clinical signs become normal, there is a gradual return of the chronaxy to its original level.

In a further study of the condition of the neuromuscular apparatus in experimental tuberculosis, we made measurements of the chronaxy and of the strength-duration curve, as well as of the lability (muscle response speed). The results are described in the present article.

METHOD

Measurements were made of the chronaxy of the flexors and extensors of the digits of the right hind feet, using a condensor chronaxiemeter and Yu. M. Uflyand's standard method [4]. To determine the strength-duration curve and the muscle response speed of the gastrocnemius muscle of the right hindleg, we used an electrical stimulator made by the State Institute of Physiotherapy (1953); it was possible to regulate the intensity of the stimulus between 0 and 200 v, and its duration from 0.01 to 100 msec, the values being read off from a scale. The threshold value of the voltage for each impulse duration was plotted on special graph paper and the strength duration curve drawn. The lability of the muscle was measured by applying pulses at rates of 1, 5, 10, 20, 50, 100, 200, 300, etc. up to 1500 impulses/second, the voltage being maintained constant at a value 1 ½ times threshold, and the duration being fixed at 0.05 msec.

The contractions were recorded on a kymograph. In one experiment, 3-7 myograms were recorded to determine lability changes occurring during the course of the reaction (adoption of a "labilization" rhythm, lability constancy). We also studied the relation between the strength of the stimulus and the strength of the contraction of the gastrocnemius muscle. Using an impulse duration of 0.05 msec, and a frequency of 50 impulses/second, we measured the response to a threshold stimulus and to stimuli 1 ½ and 2 times threshold. In healthy dogs the response of the neuromuscular apparatus before and after repeated lability measurements was proportional to the strength of the stimulus (see Fig. a_1). Equalization, or an abnormal stimulus response relationship which occurred after an infection, showed we were dealing with phasic conditions as described by N. E. Wedensky in his work on parabiosis [1]. To infect the dogs with tuberculosis, we used the usual laboratory method of intravenous injection of 1 mg per kg of a culture of bovine tuberculosis mycobacteria.

The chronaxy, strength-duration curve, and lability were measured before and after infection on 7 dogs — — Kvartus, Kutsy, Rex, Donor, Pepper, Blacky, and Dubok. Recovery of the chronaxy, strength-duration curve, and lability were studied in 4 dogs — Kvartus, Kutsy, Donor, and Pepper, because Rex and Blacky died 50-60 days after infection and Dubok was killed by electrocution for postmortem examination.

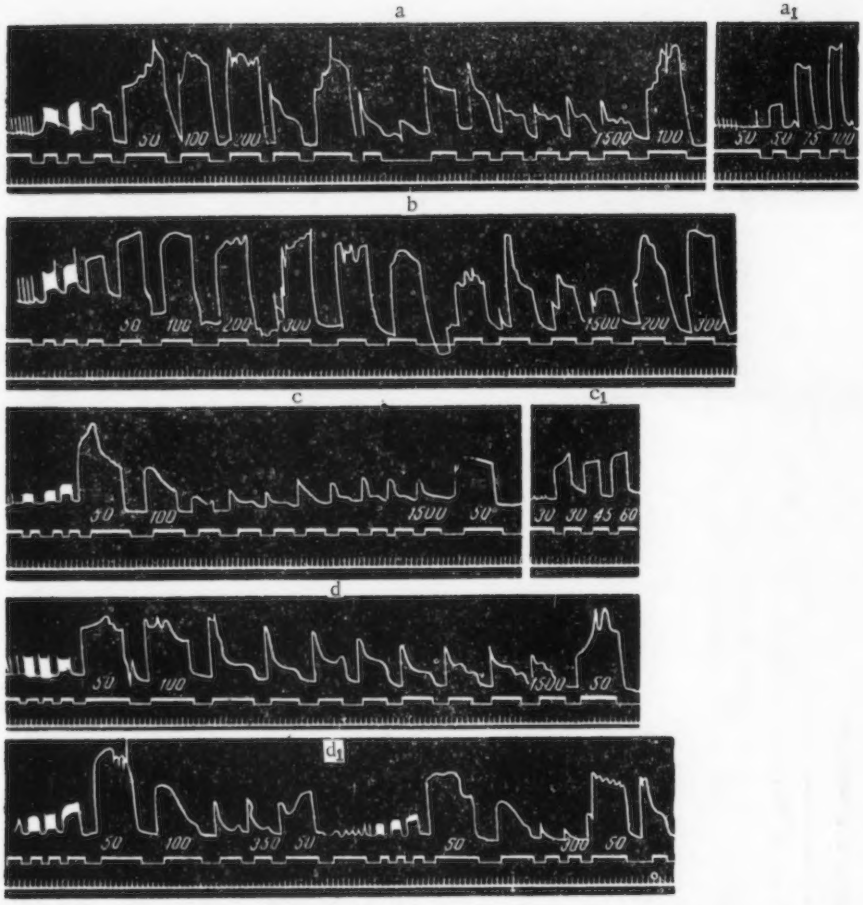

Fig. Changes in the physiological lability of the gastrocnemius muscle in experimental tuberculosis in the dog Kvartus at different times during the illness.
a) Before infection (stimulus 35 v); a_1) before infection (frequency 50 impulses/second; b) on the 7th day after infection (stimulus 15 v); c) 21 days after infection (stimulus 70 v); c_1) 21 days after infection (frequency 50 impulses/second); d – d_1) 35 days after infection (stimulus 30 v).
Curves, from above downwards: myogram; stimulus marker (frequency of impulses per second shown on myograms a, b, c, d, and d_1, and strength of stimulus in volts on myograms a_1 and c_1); time marker (1 second). Duration of impulses in all myograms 0.05 msec.

The condition of the animals which had recovered was confirmed by a positive Mantoux reaction, and in those which died the diagnosis was made from specific tuberculous changes in the lungs and pleura. On those that died – Blacky and Dukok – a test was made by culturing a portion of lung on a piece of Lowenstein's medium, when the pure culture of tuberculosis mycobacterium was grown.

We obtained 1211 myograms and 430 strength-duration curves. The condition of the dogs after infection may arbitrarily be described in terms of 4 periods: The incubational period, lasting for 4-8 days, the period of increasing clinical signs, which lasted from the 8th to the 12th day, the acute phase from the 14th to the 28th day, and recovery period (if such occurred) during the second and third months, or the terminal period if the dog died.

The first changes in the quantities measured before the manifestation of any clinical signs occurred on the 2nd-4th day after infection. The strength-duration curve was displaced towards the abscissa, and was altered in

TABLE

Changes in Physiological Lability in Dogs with Experimentally Induced Tuberculosis

Index of lability	Kvartus		Kutsy		Donor		Pepper		Dubok		Rex		Blacky	
	before infection	19 days after infection	before infection	17 days after infection	before infection	17 days after infection	before infection	19 days after infection	before infection	17 days after infection	before infection	17 days after infection	before infection	17 days after infection
Maintained tetanus	200	50	200	100	50—100	50	100	50	100	50	100	50	100	50
Decreasing tetanus.	300	100	300	200	200	100	200	100	200	100	200	100	200	100
Complete pessimum.	> 1500	1 000	> 1500	1 500	> 1 500	1 500	> 1500	1500	> 1500	300	> 1500	800	1000	300—400

shape, the change affecting principally its horizontal portion; this result indicated an increased excitability for both long and short stimuli. The chronaxy was shortened; it was determined by measurements made on the strength-duration curve, and had a value of 0.1 as opposed to the normal 0.16 msec; the value obtained by using a condensor chronaxiemeter was 0.2 msec as against a normal value of 0.28 msec.

The physiological lability was measured in terms of the following quantities: The maximal possible frequency at which a tetanus could be maintained is known as the "frequency optimum"; the frequency at which the tetanic contraction becomes reduced is known as the "frequency pessimum"; and the critical frequency is that at which minimum contraction (complete pessimum) occurs (see Fig. a, a_1, b, c, c_1, d, d_1).

Before infection, in the dogs Rex, Donor, Pepper, Dubok, and Blacky, which had chronaxies of 0.36, 0.42, 0.28, 0.28, and 0.36 msec respectively, a maintained tetanus was recorded at frequencies of 50 and 100 impulses/second, while in Kvartus and Kutsy, which had shorter chronaxies of 0.24 and 0.2 msec, the corresponding frequencies were 50, 100 and 200 impulses/second (see Fig. a). In none of the dogs did a complete pessimum develop until the frequency had risen to 1500 imp/second.

In the first days after the dogs were infected, and particularly in Kvartus, Kutsy, and Blacky, lability was increased. A steady tetanic contraction occurred only at high frequencies between 200-300 per second and a decreasing tetanic contraction only at frequencies of 400-500 per second (see Fig. b). The complete pessimum at 1500 per second never occurred.

Thus, in the first days after infection and before clinical signs had appeared, excitability was increased, chronaxy was shortened, and physiological lability increased. As the disease progressed, particularly when the condition became grave, by the 3rd or 4th week, when the temperature was 41° and the erythrocyte sedimentation rate 50 mm per hour, and the animals had lost weight, the increase in excitability and lability and the shortening and variability of the chronaxy, which had occurred in the incubation period, were no longer observed, but instead there was a progressive decrease in excitability and lability, and an increase in the chronaxy.

The strength-duration curve showed two changes, one in the horizontal and the other in the vertical portion. The horizontal knee of the curve was further removed from the abscissa, i.e., even with impulses of long duration excitability was reduced. The vertical portion of the curve was removed from the ordinate, showing that even at considerable stimulus intensities a considerable stimulus duration was required.

The chronaxy as determined from the curve was 0.27-0.4 msec, and the value measured by the condensor chronaxiemeter was 1.2-0.88 msec, showing an increase of 2-4 times.

In measuring physiological lability (see Table) during this period, in all the dogs a steady tetanus was recorded at a frequency as low as 50 impulses/second, though not invariably a decreasing tetanic contraction was also found at frequencies between 50 and 100 per second, while with stimulation rates between 300 and 400 per second or above, there was an initial tremor of the muscle, and complete pessimum soon developed (see Fig, c).

Measurements on the ratio of stimulus strength of muscle response showed that during this period the normal relationship was disturbed: In response to weak, average, and strong stimuli, the same abnormal effect occurred (equalizing and paradoxical phase – see Fig, c_1).

Subsequently, after treatment with 25 mg per kg phthivazide (a derivative of isonicotinic acid hydrazide), as the animals' clinical condition improved, it was found that the chronaxy was shortened. The strength-duration curves showed an increased excitability.

A more precise indication of the condition of the neuromuscular apparatus at this period is given by the lability: We determined the condition of the tissue not from the occurrence of a single contraction, but from many following closely on each other. Thus, in Kvartus, on the 35th day after infection, there was an increased lability in the first myogram (see Fig, d). The decreasing tetanic contraction was recorded at a frequency of 100-200 impulses/second. At a frequency of 1500 per second, no complete pessimum occurred. However, in subsequent myograms, the decreasing tetanic contraction occurred at frequencies as low as 50-100 per second, and a complete pessimum occurred at a frequency of 200-300 (see Fig, d_1). Before infection, no such changes were associated with repetition of the test; on the contrary, quite frequently during the reaction the excitatory rhythm was sometimes taken up, and the lability actually increased during the test. This effect indicates a failure to adapt to the rate of excitation, and a progressive decrease in lability during the test, and therefore a considerable reduction in the performance level of the nerve and muscle in question.

Compensation for and disappearance of the clinical signs occurred in the following 2-3 months, and were accompanied by a gradual return of excitability, shortening of chronaxy, and an increase in lability. At first the indices were unstable: At one time the chronaxy, electrical excitability, and lability were within normal limits, but periodically there would be an increased chronaxy, and a reduced excitability and lability. The indices appear to return to normal in two phases: At first they are variable, but at times come within normal limits, and later they remain stable at normal values. As a result of the complete restoration of function on the motor side, after 1-1½ months the animals became completely normal as judged by the usually accepted clinical signs.

It can be seen therefore, that tuberculosis involves profound neuromuscular changes which, from the way they develop, must be considered as manifestations of a parabiotic process. This condition may result from a central nervous disturbance, from reflex effects originating in the damaged organs, or from the direct action of the tuberculosis toxin on the motor apparatus.

From our results it would appear that the chronaxy, the shape of the strength-duration curve, and the lability as measured by the optimum and pessimum frequencies of tetanic contraction, represent important criteria for the evaluation of the condition of the neuromuscular system in tuberculosis.

SUMMARY

A study was made of the condition of the neuromuscular apparatus in dogs infected with tuberculosis. Measurements were made of the chronaxy, phsiological lability, and electrical excitability. The lability of the motor apparatus showed wide variations in healthy dogs before infection, and was greater the shorter the chronaxy.

Pronounced changes in the motor apparatus follow infection with tuberculosis. There is an increased excitability, a shortened chronaxy, and an increased lability during the incubation period. At the height of the disease there is a stable progressive decline of excitability, an increased chronaxy, a reduced lability, and an abnormal relation between stimulus and response; equalizing and paradoxical phases occur.

On recovery, the excitability, chronaxy, and lability return slowly to their initial values, and all clinical indices become normal.

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* In Russian

THE ACTION OF X-RAYS ON THE MOTOR FUNCTION AND THE CONTRACTILE PROTEINS OF MOTILE CELLS

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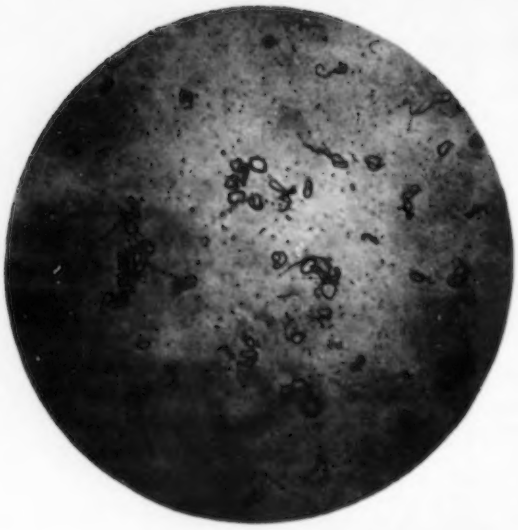
The study of the action of x-rays on the motor function of cells, and in particular of spermatozoa, is of interest from several points of view. It is possible thereby to obtain in a clearly apparent form data demonstrating that the degree of radiation injury of certain enzyme systems (for example, the thiol enzymes, the system of enzymes of oxidative phosphorylation) is determined primarily by the degree of radiosensitivity of the particular species of cell, and this, in turn, depends in the opinion of certain authors [4, 5] on the character of the intracellular localization of these enzymes.

From this point of view it can clearly be understood how marked disturbance of the processes of oxidative phosphorylation can occur in radiation sickness in a number of "radiosensitive" tissues, in the absence of any appreciable changes in these processes in muscle tissue and in other "radioresistant" organs, tissues and cells. Adult spermatozoa are an example of such radioresistant cells.

We know very well that the movement of spermatozoa is inseparably connected with continual resynthesis of adenosinetriphosphate (ATP) at the expense of energy from oxidative processes (respiration) or the anaerobic breakdown of carbohydrates [2, 3, 6, 10, 17, 18]. Any agency connected with the disturbance of the conjugation of respiration or glycolysis and the resynthesis of ATP leads to rapid loss of the motility of the spermatozoa. Thus spermatozoa, like other cells possessing the power of movement (for example the cells of ciliated epithelium), are excellent objects for the study of the association, postulated by several authors, between the development of radiation injury and the inadequate formation of high-energy compounds in the cells of irradiated animals (i.e., a fall in the intensity of oxidative phosphorylation).

The study of the influence of ionizing radiation on the motor function of cells is also of interest in other respects. The biochemical basis of the different forms of cell division is known to be the interaction between ATP and contractile protein, possessing an adenosinetriphosphatase activity and the property of changing its physical state undet the influence of ATP. A like mechanism is at the basis of muscular contraction [9, 11], the movements of the flagella of spermatozoa and of trypanosomes [6, 10, 16], the rippling of the cilia of epithelial cells [1] and the movements of the leaves of certain plants [7]. There are indications that the separation of chromosomes and the equatorial division of the protoplasm of the cell in the process of mitosis are also effected by changes in the properties of the specific contractile protein of the cytoplasm during its interaction with ATP [16, 19]. It is also known that the proteins of the actomyosin complex possess low sensitivity to ionizing radiation [5]. Since, according to Hoffmann-Berling's findings [6], the contractile proteins of different species of cell are very close in their physicochemical properties and adenosinetriphosphatase activity to actomyosin, it may be suggested that, like the proteins of the actomyosin complex, they also must possess high resistance to ionizing radiation.



Annular forms of models of spermatozoa (microphotograph).

From considerations such as these it might be possible to explain the high resistance of certain phases of cell division [15], for example the interphase and anaphase, which is due to interaction between the contractile cell proteins and the ATP.

In the present communication we describe the results of research into the effect of massive doses of x-rays on the motor function of living spermatozoa and on the reaction between adenosinetriphosphate and "glycerol models" of myofibrils, spermatozoa and the cells of ciliated epithelium.

EXPERIMENTAL METHOD

Experiments were carried out on male spring frogs, which were given a single exposure to x-rays in a dose of 20,000 r. Conditions of irradiation: time of exposure – 61 minutes, voltage – – 185 kv, current – 15 ma. Spermatozoa from the testicles of the irradiated frogs were suspended in a 0.1% solution of NaCl. Their motility was examined under the microscope.

The "glycerol models" of spermatozoa were obtained by Hoffmann-Berling's [16] method, which we simplified in accordance with the findings of Aleksandrov and Arronet [1], which was essentially as follows: Minced tissue from the testicles of a sacrificed frog was immersed in 2-3 volumes of a 50% aqueous solution of glycerol and placed in the refrigerator for 24 hours; one drop of the glycerol suspension was then mixed with 2-3 drops of a 0.12 M solution of KCl, 0.01 M phosphate buffer (pH = 7.0) and 0.005 M $MgCl_2$; a few minutes later one drop of a neutralized 0.01 M solution of ATP was added to the suspension of spermatozoa thus obtained, on a glass slide, and the action of the ATP on the "cell models" of the spermatozoa, or more accurately, on the "cell cadavers", was studied under the microscope.

The muscle fibers were extracted with a 50% glycerol solution by the method described by Bendall [14].

Preparations of the cilia of the soft palate were made by the method of Aleksandrov and Arronet [1].

EXPERIMENTAL RESULTS

Living spermatozoa from frogs irradiated with 20,000 r retained good motility in isotonic saline solutions, and in this respect were not appreciably different from the spermatozoa of unirradiated control animals.

The preservation of the motility of living spermatozoa obtained from the testicles after irradiation of frogs with massive doses of x-rays demonstrated the radioresistance of the mechanisms maintaining the ATP at the level necessary for effecting the motor function. These experiments therefore gave no grounds for postulating in this case any disturbance of oxidative phosphorylation under the influence of ionizing radiation. This in no way implies that if the distribution of the enzymes catalyzing the processes of oxidative phosphorylation (in radiosensitive tissues) were of a different character, the oxidative resynthesis of ATP would not be disturbed also. On the contrary, from the general considerations outlined above and from the reports in the literature [5], the opposite may be declared.

After the addition of ATP and a solution containing $MgCl_2$, the spermatozoa of the frog, extracted with glycerol, often change their shape: 65-70% of the spermatozoa form "annular" structures (see figure); many of them perform energetic oscillatory movements with their tails. In some cases, after extraction with glycerol, solitary motile spermatozoa were found even after the addition of saline without ATP; in these cases, however, the motility disappeared completely after 15-20 minutes and reappeared after additon of a 0.01 M solution of ATP to the suspension. In these cases the reappearance of motility in the spermatozoa extracted with glycerol without the addition of ATP was probably explained by the presence of a small amount of endogenous, protein-

-bound ATP, which was quickly used up after the addition of the saline solution containing $MgCl_2$ which activated the utilization of ATP by the spermatozoa.

The "glycerol models" of the spermatozoa of animals irradiated with 20,000 r preserved their motility under the influence of ATP and $MgCl_2$ ions introduced from outside.

The contractility of the muscle fibers of frogs, irradiated with massive doses of x-rays, when treated with glycerol, did not differ appreciably after the addition of ATP from the contractility of the fibers of unirradiated animals. For instance, the myofibrils obtained from the gastrocnemius muscle of the irradiated animals reacted to ATP by a shortening of 50-70% of their length, and the myofibrils of the unirradiated frogs by a shortening of 55-60%. The rate of contraction, too, was roughly the same in both cases.

The experiments with preparations of the soft palate of frogs, extracted with 50% glycerol solution, showed that the cilia of the cells of the ciliated epithelium of animals irradiated with 20,000 r retained the power of rippling movement after the addition of ATP and $MgCl_2$.

The results of the experiments on "glycerol models" thus showed that the contractile proteins of cells possess radioresistance and do not lose their power to interact with ATP under the influence of massive doses of ionizing radiation.

The facts which we have described, together with indications in the literature on the common structural and functional features of the different contractile proteins, suggest that the low sensitivity of certain phases of cell division (interphase, anaphase, associated with separation of the chromosomes and equatorial division of the cytoplasm) to ionizing radiation is also explained by the radioresistance of contractile protein, which is responsible for the cytokinesis of the dividing cells.

The preservation of the contractile properties of these proteins and of their power to interact with ATP after irradiation even with massive doses of x-rays indicates the radioresistance of those SH-groups in the protein molecule which give it its adenosinetriphosphatase character [12, 8]. This is evidence against Barron's theory that the primary biological action of ionizing radiation is connected with the inactivation of the thiol enzyme systems and is unconcerned with their intracellular localization [13].

The findings described above are in agreement with direct observations indicating the absence of any essential lowering of the content of SH-groups in the proteins of the actomyosin complex or in the serum proteins in response to the action of minimal lethal doses of x-rays on animals [5].

SUMMARY

Experiments showed that there are no significant changes of the motor function in the seminal cells after x-ray irradiation of frogs (20,000 r). This shows that disturbance of oxidative phosphorylation under the action of radiation is not a general biological regularity. The effect of ionizing radiation on the resynthesis of high-energy (ATP) differs in the cells of radiosensitive and radioresistant tissues; evidently it is primarily determined by the different localization of the corresponding enzyme systems therein.

There are no visible disturbances in the ability of "glycerol models" of myofibrils, the cilia of the ciliated epithelium and spermatozoa (obtained from the tissues of irradiated animals), to interact with ATP. This points to a rather low radiosensitivity of the contractile cellular proteins and explains the high radioresistance in some phases of cell division connected with the separation of the chromosomes and equatorial division of the cytoplasm.

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THE EFFECT OF PACKING OF THE RED CELLS ON THE ESR

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The elucidation of the laws governing the sedimentation of the red cells helps toward an understanding of the ESR, a reaction widely used in the practice of medicine and one of considerable diagnostic value.

Among the various factors influencing the rate of sedimentation of the red cells, we may pick out the packing of the red cells. This factor is constantly present in any blood; it plays an essential role in the process of sedimentation of the red cells and appreciably affects the character of the curve of sedimentation of the red cells with time. In the course of sedimentation, as the column of red cells shrinks, their density increases and this interferes with further sedimentation. This factor, which at first is of little consequence, acquires ever increasing importance until the end of the process.

Many processes are characterized by the same feature: with a change in a certain parameter, its further change in the same direction is rendered more difficult. All such processes obey an exponential relationship. We considered it to be possible to consider the changes in the sedimentation of red cells with time as an exponential relationship also. We shall derive a mathematical relationship between the value of the ESR and time.

It can be assumed that

$$v = \frac{c}{\rho}, \tag{1}$$

where v is the rate of sedimentation of the red cells, ρ the viscosity of the blood, and c is a coefficient.

Now

$$\rho = \frac{y}{h}, \tag{2}$$

where y is the relative viscosity of the blood and h the height of the column of red cells at given time t.

Substituting the value of ρ from equation (2) in (1), we find

$$v = \frac{c}{y} h$$

or, combining c and y in one coefficient k, specific for each sample of blood, we obtain

$$V = kh. \tag{3}$$

But the height of the column of red cells

$$h = H - S, \tag{4}$$

where H is the initial height of the column of blood (in a capillary tube H = 100 mm), and S is the height of the column of plasma, i.e., the value of the ESR itself.

Therefore,

$$v = k\,(H - S). \tag{5}$$

Since

$$v = \frac{dS}{dt}, \text{ then } \frac{dS}{dt} = k\,(H - S). \tag{6}$$

Transposing, we have

$$\frac{dS}{H-S} = k dt. \tag{7}$$

Since

$$d(H - S) = 0 - dS, \text{ then}$$
$$dS = -d(H - S).$$

Substituting in equation (7), we obtain

$$-\frac{d(H-S)}{H-S} = k dt. \tag{8}$$

Integrating,

$$-\ln(H - S) = kt + k_2 \tag{9}$$

Substitute

$$k_2 = -\ln k_3,$$

Then

$$\ln(H - S) + \ln k_3 = -kt. \tag{10}$$

Potentiating equation (10):

$$k_3(H - S) = e^{-kt}; \quad H - S = \frac{1}{k_3} e^{-kt}; \quad S = H - k_4 e^{-kt}. \tag{11}$$

Let us deduce the value of k_4. When t = O and S = O, k = H.

Consequently,

$$S = H(1 - e^{-kt}). \tag{12}$$

As the height in equation (12) we must not use the initial height of the column of blood but that of the column of plasma over the fully sedimented red cells in these conditions:

$$S_0 = H - h_0, \tag{13}$$

where h_0 is a constant, unchanged in the particular conditions of sedimentation of the column of red cells.

Consequently,

$$S = S_0 (1 - e^{-kt}). \tag{14}$$

In order to test the applicability of the theoretically derived equation (14) to the ESR – time curve obtained experimentally, we carried out experiments in which the sedimentation in 24 samples of blood was measured by the usual technique every 15 minutes for a period of 2 hours and then at less frequent intervals for 48 hours. At this time the process of sedimentation was practically complete, and a column of red cells of constant height h_0 was obtained; knowing the height of this column, we could calculate the coefficient k for this blood, using an arbitrarily selected value of S at any one of the times at which measurements were taken. Having determined k, the theoretical values of S could be calculated (S_T). We compared these with the experimental values S_{exp}.

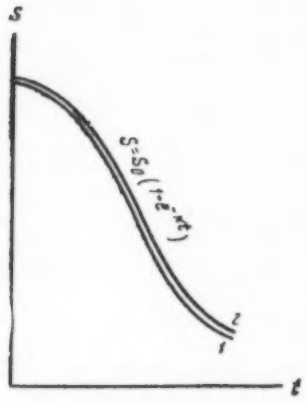

Fig. 1. Experimental (1) and theoretical (2) ESR – time curve when the ESR is only slightly raised.

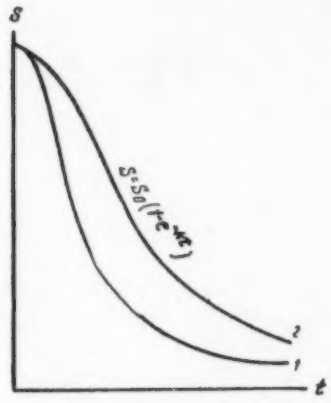

Fig. 2. Experimental (1) and theoretical (2) ESR – time curve when the ESR is raised.

The applicability of equation (14) to the ESR – time curves was tested in the 24 experiments. Examples of certain changes in the sedimentation of red cells with time, as determined experimentally, are compared in the table with the values of S_T calculated theoretically from equation (14). The comparison showed that in the majority of cases when the ESR was normal or slightly raised, the theoretical values S_T agreed reasonably closely with the values S_{exp} obtained experimentally (of the 15 ESR – time curves 14 showed close agreement and one rather more distant agreement). Conversely, in the majority of cases when the ESR was raised, essential discrepancies were observed between the theoretical and experimental values, these being most pronounced in the initial stage of the ESR–time curve. Of 9 cases in which the ESR was raised (from 13 to 56 mm in 1 hr) close agreement was obtained in only 2 cases, and more distant in seven.

We account for the results obtained as follows. The initial portion of the ESR – time curve shows individual peculiarities. It owes its character mainly to the degree of agglomeration of the red cells, and this depends in particular, on the state of the body, and it is increased in pathological processes when the sedimentation rate of the red cells is raised. The final portion is common to any ESR – time curve. It mainly depends on the number of red cells in the blood. When the ESR is nor raised, the degree of agglomeration of the red cells is smaller; it produces no great deviation from the theoretical curve, for which the equation contains no agglomeration factor; the experimental and theoretical ESR – time curves agree (Fig. 1). Conversely, when this agglomeration

TABLE

Applicability of the Equation $S = S_0 (1 - e^{-kt})$ to the ESR – time Curve

Indices	Time (in minutes)	Sedimentation		Difference
		experimental	theoretical	
		in mm		
Cases of close agreement				
1. Limiting sedimentation $S_0 = 35$ mm; time chosen for finding k, $t_k = 720$; sedimentation at time t_k, $S_k = 28$ mm; $k = 0.00219$	15	0,5	1.2	+0.7
	30	1	2.2	+1.2
	45	3	3.4	+0.4
	60	4	4.3	+0.3
	75	5	5.9	+0.9
	90	7	6.3	−0.7
	105	8	7.2	−0.8
	120	9	8.1	−0.9
	720	28	27.8	−0.2
	1 440	32	33.6	+1.6
	2 880	35	34.9	−0.1
2. $S_0 = 56$ mm; $t_\kappa = 120$; $S_\kappa = 32$ mm; $\kappa = 0.007$	15	2	5.6	+3.6
	30	6	10.7	+4.3
	45	10	15.8	+5.8
	60	17	19.3	+2.3
	75	2	22.8	+0.8
	90	25	26.2	+1.2
	105	28	29.1	+1.1
	120	32	31.9	−0.1
	135	34	34.2	+0.2
	150	36	35.5	−0.5
	165	37	38.4	+1.4
	180	38	40.1	+2.1
	420	46	52.6	+6.6
	720	50	55.4	+5.4
	1 440	53	56.9	+3.9
	1 880	55	56.9	+1.9
Cases of more distant agreement				
3. $S_0 = 60$ mm; $t_\kappa = 720$; $S_\kappa = 56$ mm; $\kappa = 0.00375$	15	3	3.3	+0.3
	30	10	6.4	−3.6
	45	19	9.2	−9.8
	60	25	12.0	−13.0
	75	32	14.6	−17.4
	90	37	17.2	−19.8
	105	40	19.5	−20.5
	120	41	21.8	−19.2
	720	56	56.0	0.0
	1 440	58	59.6	+1.6
	2 880	60	59.9	−0.1

is considerable (which is more common when the ESR is raised), a considerable divergence is observed between the experimental and theoretical ESR – time curves, for the latter takes no account of the agglomeration factor (Fig. 2).

The experimental results thus show that packing of the red cells in many cases considerably affects the

ESR – time curve. As a rule this is of no clinical importance. Packing of the red cells, however, conceals and distorts the manifestation of agglomeration of the red cells, which alters the ESR in accordance with the state of the body. It is therefore desirable to develop a method of estimation of the ESR in which packing of the red cells would not take place or would be minimized.

SUMMARY

As the sedimentation of red cells goes on they become increasingly compact, which hinders their further sedimentation. This enables one to apply the exponential relation $S = S_0(1-e^{-kt})$ to the ESR – time curve.

Experimental verification of the formula has demonstrated that with a normal and slightly accelerated ESR there is a good accord between the experimental and the theoretical sedimentation values, while in the event of increased ESR, there is considerable discrepancy between them.

This is caused by the absence of the factor of the red cell agglomeration in the formula, which is of decisive significance in the acceleration of ESR.

PHARMACOLOGY

THE IMPORTANCE OF THE POSITION OF THE QUATERNARY NITROGEN ATOM IN THE MOLECULE OF CERTAIN COMPOUNDS TO THEIR ABILITY TO REACT WITH TRUE CHOLINESTERASE AND PSEUDOCHOLINESTERASE

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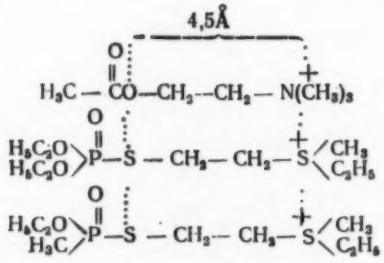
The development of a free positive charge on the nitrogen atom during the alkylation of the tertiary amines usually leads to enhancement of the biological action of the cholinolytic, cholinomimetic and anticholinesterase compounds (for the literature on this subject, see [1, 2]). The development of a positive charge on the sulfur or nitrogen atom enhances the anticholinesterase action of certain organic phorphorus compounds [3, 4, 5, 6, 8, 9]. If the structure of these compounds is compared with the structure of certain other compounds affecting the function of cholinergic structures, attention is drawn to the fact that the distance between the central atom of the cationic part of the molecule and its ester oxygen (sulfur) atom is identical both in the cholinesterase inhibitors under study and in acetylcholine.



Acetylcholine

Isosystox methylate

Preparation Gd-42*

In acetylcholine this distance (~ 4.5 Å)** must evidently correspond to the distance between the anionic and esterase points of the active center of the cholinesterase [10]. The strengthening of the biological activity of organic phosphorus compounds after the development of a charge in the molecule must be based on the acquisition by the compounds of the ability to become rapidly orientated on the surface of the cholinesterase. The

*Isosystox methylate was synthesized by T. A. Mastryukova, and preparation Gd-42 by N. N. Godovikova, in M. I. Kabachnik's laboratory (INEOS AN SSSR).

**Here and subsequently the distances (4.5Å and 6 Å) are given as the sums of the interatomic distances.

TABLE 1

Rate of Hydrolysis of Certain Tertiary Amines and Their Methiodides with Pseudocholinesterase

No.	Structure of preparation*	R	Distance R---O- (in A)	Rate of hydrolysis of prep. with 1 unit of enzyme (in mM/hr)	Change in rate after acquisition of charge
1	R—CH_2—CH_2—O—C(=O)—$C(CH_3)_2 \cdot C_6H_5$	$-N(CH_3)_2 \cdot HCl$	4.5	$1 \cdot 10^{-3}$	×4.2
2		$-\overset{+}{N}(CH_3)_3 \cdot \overset{-}{I}$		$4.2 \cdot 10^{-2}$	
3		$-N(C_2H_5)_2 \cdot HCl$	4.5	$8.5 \cdot 10^{-3}$	×3.8
4		$-\overset{+}{N}(C_2H_5)_2CH_3 \cdot \overset{-}{I}$		$3.2 \cdot 10^{-2}$	
5	R—CH_2—CH_2—CH_2—O—C(=O)—$C(CH_3)_2 \cdot C_6H_5$	$-N(CH_3)_2 \cdot HCl$	6.0	$9.5 \cdot 10^{-4}$	No hydrolysis occurs
6		$-\overset{+}{N}(CH_3)_3 \cdot \overset{-}{I}$		0	
7		$-N(C_2H_5)_2 \cdot HCl$	6.0	$9 \cdot 10^{-4}$	The same
8		$-\overset{+}{N}(C_2H_5)_2CH_3 \cdot \overset{-}{I}$		0	

* The preparations were synthesized by G. T. Tatevosyan and S. Agbalyan in the Insitute of Fine Organic Chemistry of the AN Armenian SSR, Erevan (Director of the Institute: A. L. Mndzhoyan).

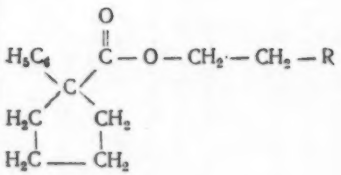
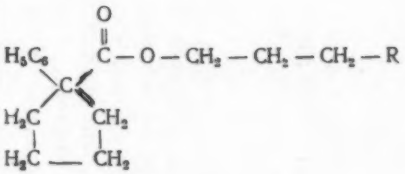
positively charged atom of the molecule is, as it were, anchored to the anionic point of the active center of the enzyme, and begins to revolve in a narrow orbit. The probability of collision between the phosphorylating group of the inhibitor and the esterase point of the active center of the cholinesterase, necessary for its phosphorylation, thus becomes increased.

If this hypothesis is correct, the strengthening of the power to react with cholinesterases by the development of a positive charge in the molecule of the inhibitor will probably depend on the distance from the atom of oxygen or sulfur) forming the ester bond to that in which the charge appeared. If this distance corresponds to the distance between the anionic and esterase points of the active center of the cholinesterase, maximum increase in activity may be expected.

TABLE 2

Anticholinesterase Action of Certain Tertiary Amines and Their Alkylates

No.	Structure of preparation*	R	Distance R---O (in A)	Cholinesterase			
				pseudo-		true	
				I_{50}(M)	change in action after alkylation	I_{50} (M)	change in action after alkylation
9	$(C_6H_5)_2CH-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-R$	$-N(C_2H_5)_2 \cdot HCl$	4.5	$1 \cdot 10^{-4}$	× 48	$2 \cdot 10^{-3}$	1
10		$-\overset{+}{N}(C_2H_5)_3 \cdot \overline{I}$		$2.1 \cdot 10^{-6}$		$1.9 \cdot 10^{-3}$	
11	$(C_6H_5)_2CH-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-CH_2-R$	$-N(C_2H_5)_2 \cdot HCl$	6.0	$8.2 \cdot 10^{-6}$	: 1.6	$2.2 \cdot 10^{-3}$	:5.9
12		$-\overset{+}{N}(C_2H_5)_2CH_2 \cdot \overline{OSO_2OCH_3}$		$1.3 \cdot 10^{-5}$		$1.3 \cdot 10^{-2}$	
13	$C_6H_5(nC_3H_7)_2C-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-R$	$-N(CH_3)_2 \cdot HCl$	4.5	$9.2 \cdot 10^{-4}$	× 115	$1.8 \cdot 10^{-3}$	×3.9
14		$-\overset{+}{N}(CH_3)_3 \cdot \overline{I}$		$8 \cdot 10^{-6}$		$4.6 \cdot 10^{-4}$	
15	$C_6H_5(nC_3H_7)_2C-\overset{O}{\overset{\|}{C}}-O-CH_2-CH_2-CH_2-R$	—	6.0	$5.9 \cdot 10^{-4}$	: 1.3	$2.3 \cdot 10^{-3}$	: 1.5
16		$-\overset{+}{N}(CH_3)_3 \cdot \overline{I}$		$7.4 \cdot 10^{-4}$		$3.4 \cdot 10^{-3}$	
17	$\begin{matrix}CH_3\\ \vert\\ CH-CH_2-O-C_6H_4-\overset{O}{\overset{\|}{C}}-O-\underset{\vert}{\overset{CH_3}{CH}}-\underset{\vert}{\overset{CH_3}{CH}}-CH_2-R\\ \vert\\ CH_3\end{matrix}$	$-N(C_2H_5)_2 \cdot HCl$	6.0	$1.9 \cdot 10^{-5}$	1	$2.4 \cdot 10^{-4}$	:3.4
18		$-\overset{+}{N}(C_2H_5)_2CH_3 \cdot \overline{I}$		$1.9 \cdot 10^{-5}$		$8.4 \cdot 10^{-4}$	

19		—N $(C_2H_5)_2 \cdot HCl$	4.5	$1.2 \cdot 10^{-4}$	× 67	$2.5 \cdot 10^{-3}$	× 11
20		—$\overset{+}{N}(C_2H_5)_2\,CH_3 \cdot \overset{-}{S}O_4CH_3$		$1.8 \cdot 10^{-6}$		$2.2 \cdot 10^{-4}$	
21		—N $(C_2H_5)_2 \cdot HCl$	6.0	$1.5 \cdot 10^{-6}$	: 13.3	$2.4 \cdot 10^{-3}$	× 1.6
22		—$\overset{+}{N}(C_2H_5)_2\,CH_3 \cdot \overset{-}{S}O_4CH_3$		$2 \cdot 10^{-5}$		$1.5 \cdot 10^{-3}$	

*Synthesis of the preparations was as follows: No. 10 by N. L. Kudryashova (IÉM, Leningrad); Nos. 11 and 12 by A. L. Mndzhoyan and O. L. Mndzhoyan; Nos. 13, 14, 15 and 16 by G. T. Tatevosyan and S. Agbadyan; Nos. 17 and 18 by V. D. Afrikyan and M. Grigoryan (Institute of Fine Organic Chemistry AN Armenian SSR, Erevan); No. 20 by N. V. Khromov-Borisov and A. M. Yanovitskaya (First Leningrad Medical Institute).

We had at our disposal a group of pseudocholinesterase substrates and a group of selective inhibitors of the enzyme, containing a nitrogen atom in their molecules, and we tried to use these preparations to show how the development of a positive charge at different distances from the oxygen atom forming the ester bond affected the power of the compounds to react with the enzyme: at a distance of about 4.5 Å in esters of alkylaminoethanol and at a distance of about 6 Å in esters of alkylaminopropanol.

Experiments with cholinesterase substrates. We investigated the rate of enzymic hydrolysis of 8 phenyldimethylacetic esters of choline, homocholine, and their diethylamine analogues with a purified and 40 times concentrated pseudocholinesterase of horse serum (Table 1). The determinations were made by an indicator method, namely by the rate of decolorization of phenol red by phenyldimethylacetic acid, given off in the process of hydrolysis of the compounds. Each sample was made up as follows: 0.2 ml of an aqueous solution of the cholinesterase preparation; 2.7 ml of bicarbonate buffer (pH = 7.9); 0.1 ml of a 0.025% solution of phenyl red in a borate buffer (pH = 8.0); 1 ml of a solution of the test preparation in a concentration of $1 : 10^{-2}$ M. The reactions were conducted at a temperature of 19-20°, in the chamber of a photoelectric colorimeter type FÉK-M, with a green color filter transmitting the band 5200-5400 Å (chamber 5.060 mm). Records were made of the changes in extinction (ΔE) in unit time t. Observations were continued until 7-10 reliable points had been obtained in a rectangular system of coordinates (ordinate – E, abscissa – time of experiment).

In order to determine the relationship between ΔE and the amount of phenyldimethylacetic acid (i.e., the amount of preparation decomposed), we performed two experiments in which the rate of hydrolysis of the compounds was determined concurrently by potentiometric titration and by the indicator method. This enabled the conversion coefficient for obtaining the amount of substrate hydrolyzed in mM/t from the value of $\Delta E/t$ to be determined.

It will be seen from Table 1, in which the results of the experiments are given, that methylation of the nitrogen atom situated at a distance of ~ 4.5 Å from the oxygen atom of the ester bond led in both cases to an approximately fourfold increase in the rate of hydrolysis. Conversely, in the two cases when the distance from the nitrogen atom to the oxygen atom of the ester bond was increased ~ 6 Å, methylation led to a complete cessation of detectable hydrolysis of the preparations. Hydrolysis of the preparations also ceased as the result of the addition of the cholinesterase inhibitors proserine and phosphacol to the test sample. This is evidence in favor of hydrolysis of the preparations being effected by cholinesterase itself.

Experiments with cholinesterase inhibitors. We determined the molar concentrations of a number of compounds (Table 2) which, in vitro, caused inhibition of the activity of true cholinesterase and pseudocholinesterase to the extent of 50% (I_{50}). Experiments were carried out by Hestrin's method [7]. The composition of each test sample was as follows: 1 ml of a 1.10^{-3} aqueous solution of acetylcholine chloride, 1 ml of the source of cholinesterase, 1 ml of an aqueous solution of the test compound and 1 ml of distilled water. The reaction of hydrolysis of acetylcholine was conducted at a temperature of 38 ± 0.25°. The enzymic hydrolysis of acetylcholine was terminated at the conclusion of the given exposure period (usually 120 seconds) by pouring 1 ml of a 15-20% solution of trichloroacetic acid into the flask.

As a source of true cholinesterase we used defibrinated ox blood, and as a source of pseudocholinesterase – – horse serum. The concentration of acethydroxamic acid formed as a result of Hestrin's reaction was estimated in the FÉK-M photoelectric colorimeter with a 5.060 mm chamber, using a green color filter transmitting a waveband of 5200-5400 Å.

Table 2, which gives the results of this series of experiments, shows that all the preparations investigated were more effective as inhibitors of pseudocholinesterase and showed some variable degree of selectivity. The most informative results were therefore those obtained with this enzyme, especially as the action of the preparations on the two cholinesterases was affected differently by alkylation. In all cases when the distance between the nitrogen atom and the oxygen atom of the ester bond was 4.5 Å, alkylation led to enhancement of the power of the preparations to inhibit cholinesterase. If this distance increased to 6 Å, then after alkylation of the nitrogen atom, the anticholinesterase action, on the contrary, was slightly weakened or, in any case, remained unchanged.

The exception to this was difacil (trasentin), in which, after alkylation, the inhibitory action on true cholinesterase showed practically no increase (see Table 2, Nos. 9 and 10). In this connection it is important to mention that all the compounds studied inhibited true cholinesterase only very weakly and that their action in this enzyme was generally speaking little affected as a result of alkylation.

In the course of the work we checked the rate of hydrolysis of the preparations by the cholinesterases by means of the indicator method. The rate of their enzymic decomposition in our experimental conditions was very low and could not appreciably affect the results of the investigation of the anticholinesterase action.

The results obtained may be understood on the basis of the hypothesis that the distance between the anionic and esterase points of the active center in the molecules of the cholinesterases corresponds to the distance between the nitrogen atom carrying the positive charge and the oxygen atom of the ester bond in the acetylcholine molecule. The ability of the compounds to react with the cholinesterases may, therefore, be increased by the attachment of a positive charge to the molecule only when this charge is present in the molecule at an adequate distance from the oxygen atom of the ester bond.

SUMMARY

It thus follows that, in order to increase the power of cholinesterase substrates and inhibitors to react with these enzymes, great importance is attached not only to the development of a positive charge in the molecule of the compound, but also to the distance between the atom carrying the charge and the atom forming the ester bond in the molecule. In the group of esters of some organic acids and alkylaminoalcohols which were investigated, the appearance of the charge intensified this interaction only when the oxygen atom of the bond was at the same distance from the positively charged nitrogen atom as in the acetycholine molecule. With increase of this distance the appearance of the charge weakened the ability of the majority of the substances to react.

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THE EFFECT OF PAPAVERINE, EUPHYLLINE, AND DIBAZOL ON THE RATE OF THE CORONARY CIRCULATION, THE OXYGEN DEMAND OF THE HEART, AND THE WORK OF THE HEART

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In view of the widespread occurrence of diseases associated with disturbances of the coronary circulation, the experimental evaluation of drugs used in the treatment of coronary insufficiency is of great interest.

The literature contains a number of papers dealing with the study of the effect of papaverine, euphylline, and dibazol on the rate of the coronary blood flow [2, 5, 6, 8, 9, 10]. However, a sufficiently full idea of the character of the effect of these drugs on the blood supply of the heart can only be obtained if the oxygen demand of the heart and the work of the heart are studied at the same time as measurements are made of the rate of the coronary circulation.

The aim of the present research was to investigate the influence of papaverine, euphylline, and dibazol on the coronary circulation. As criteria of the state of the blood supply of the heart, we used the volume velocity of the coronary blood flow, the oxygen demand of the heart, and the work of the heart.

EXPERIMENTAL METHOD

Investigations were carried out on cats anesthetized with nembutal (40 mg/kg). While artificial respiration was applied to the animals, the chest was opened and a polyethylene catheter was introduced through the right auricle into the coronary sinus. Clotting of the blood was prevented by heparin (1500 units/kg). The rate of the coronary blood flow was judged by the volume of blood leaving the coronary sinus in a given interval of time. A detailed description of the method is given in the paper by N. V. Kaverina [1].

Concurrently with the determination of the volume velocity of the blood flow, the oxyhemoglobin concentration in the coronary venous blood flowing through the catheter was measured with an oxymeter. Parallel recordings were made of the oxyhemoglobin concentration in the arterial blood and of its hemoglobin content. The results obtained were used to determine the oxygen demand of the cardiac muscle. We described the method of measurement of the oxygen demand of the heart in our previous communication [3].

In a group of experiments the minute volume of the heart was also recorded along with measurements of the volume velocity of the flow of blood from the coronary sinus and of the oxygen demand of the heart. This was done by means of a special apparatus allowing the volume velocity of the blood flow in the pulmonary artery to be determined periodically [4].

The arterial pressure in the carotid artery was recorded with a mercury manometer. The figures obtained

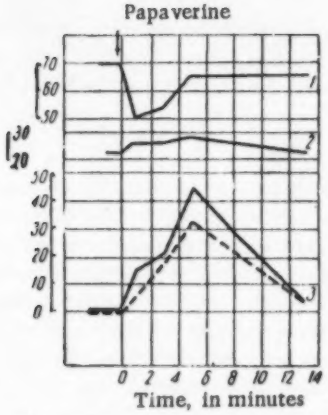

Fig. 1. The influence of papaverine on the blood supply of the heart. 1) Arterial pressure in mm Hg; 2) oxygen content in the blood of the coronary sinus in % of oxyhemoglobin; 3) outflow of blood from the coronary sinus as % of the initial level; ——) uptake of oxygen from the blood passing through the coronary sinus by the heart muscle, as % of the initial level - - - ; the arrow indicates the injection of papaverine in a dose of 1 mg/kg.

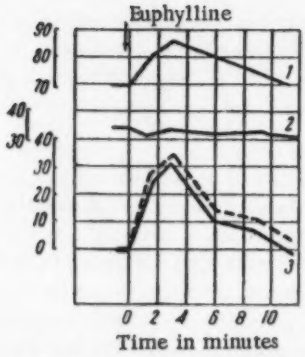

Fig. 2. The effect of euphylline (3 mg/kg) on the blood supply of the heart. Legend as in Fig. 1.

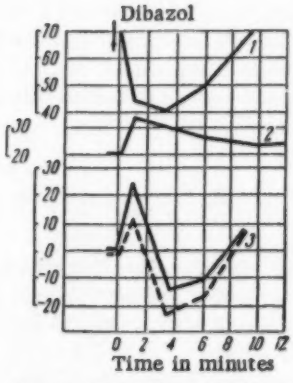

Fig. 3. The effect of dibazol (10 mg/kg) on the blood supply of the heart. Legend as in Fig. 1.

for the arterial pressure and the minute volume were used for calculation of the work of the left side of the heart. In view of the fact that the blood passing through the coronary sinus supplies mainly the left ventricle, we took into consideration only the work of the left side of the heart.

The drugs investigated were those most frequently used in the treatment of coronary insufficiency: papaverine (0.5, 1.0, and 2.0 mg/kg), euphylline (1, 3, 5, and 10 mg/kg) and dibazol (1, 3, 5, and 10 mg/kg). They were injected intravenously. Fifty experiments were carried out.

EXPERIMENTAL RESULTS

Experiments with papaverine. As a result of investigations it was shown that papaverine has the most pronounced influence on the rate of the coronary circulation. In a dose of 2 mg/kg, papaverine caused the volume velocity of the coronary blood flow to increase by an average of 70%, its duration of action being 45 minutes. Detailed information on the influence of papaverine on the rate of the coronary circulation has been given by us previously [2].

Measurements of the oxygen uptake of the heart showed that the increase in the volume velocity of the coronary blood flow was invariably accompanied by a corresponding increase in the oxygen demand of the cardiac muscle. As may be seen from Fig. 1, the curves of oxygen uptake and of the rate of flow of blood from the coronary sinus were almost parallel. The increase in the volume velocity of the coronary blood flow characteristically exceeded the increase in the oxygen demand of the heart to an insignificant degree. In the majority of experiments, papaverine caused an increase of 3-5% of oxyhemoglobin in the oxygen content of the blood of the coronary sinus (Fig. 1).

It must be pointed out that the curve of the oxyhemoglobin content of the blood in the coronary sinus reflects the correspondence between the rate of the coronary blood flow and the oxygen demand of the cardiac muscle. If the volume velocity of the coronary blood flow increased more than the oxygen uptake by the heart (see Fig. 1), the percentage of oxyhemoglobin in the blood of the coronary sinus rose. In cases when the increase

in the oxygen demand by the heart muscle preceded the increase in the rate of the coronary circulation, the percentage of oxyhemoglobin in the out-flowing blood was lowered. The character of the change in the oxyhemoglobin level in the blood of the coronary sinus in the papaverine experiments, showed that the increase in the rate of the coronary blood flow under the influence of papaverine slightly exceeded the uptake of oxygen by the cardiac muscle.

Since the ability of papaverine to increase the oxygen demand of the heart was unexpected, we conducted experiments to study the influence of papaverine on the oxygen demand of cardiac muscle by Warburg's method. For this purpose, in cats under artificial respiration the chest was opened and a strip of myocardium, weighing about 50 mg, was excised from the apex of the heart. Next, papaverine was injected intravenously in a dose of 1-3 mg/kg; 2-3 minutes later a second strip of myocardium was excised. Weighed samples of the two strips of myocardium were placed in a Warburg's apparatus*. The findings thus obtained confirmed the results of the experiments on the intact animal. The intensity of uptake of oxygen by the weighed samples of myocardium excised after the injection of papaverine was 21-41% greater, after incubation for 40 minutes, than the uptake of oxygen by the control samples.

It should be mentioned that the arterial pressure was lowered by the action of papaverine. The duration and magnitude of the depressor effect depended on the size of the dose of papaverine. In a dose of 0.5 mg/kg, papaverine lowered the arterial pressure by 15-30 mm Hg and its effect lasted from 3 to 10 minutes; in a dose of 2 mg/kg it lowered the arterial pressure by 25-45 mm for a period of 4-20 minutes. Very often, immediately after the depressor reaction an increase in the arterial pressure by 10-20 mm Hg above the initial level was observed. In some experiments, the increase in the rate of the coronary blood flow and in the oxygen demand of the heart under the influence of papaverine were maximal during the period of raised arterial pressure.

Measurements of the minute volume of the heart showed that this rose immediately after injection of papaverine, although the arterial pressure was lowered. In one of the experiments, for instance, 2 minutes after injection of a dose of 0.7 mg/kg of papaverine the arterial pressure fell from 90 to 70 mm Hg, but the minute volume rose from 133 to 166 ml. A similar relationship between the changes in the arterial pressure and minute volume was obtained in 5 of the 7 experiments. In 2 experiments the minute volume of the heart fell after injection of papaverine; the fall in the arterial pressure in these cases was sharp.

The work of the left heart, as a rule, was diminished after injection of papaverine. We were unable to detect any definite relationship between the changes in the work of the heart and the changes in the coronary circulation due to papaverine.

The influence of papaverine on the heart rate was insignificant. Immediately after injection of papaverine in a dose of 1.5-1.0 mg/kg, a slight fall in the heart rate was observed (by 5-6%), and 8-10 minutes later, this was replaced by a slight increase (by 3-4%). After injection of smaller doses of papaverine (0.5 mg/kg) a slight increase in the heart rate was observed (by 4-6%).

Experiments with euphylline. These experiments showed that euphylline had a marked effect on the volume velocity of the coronary blood flow. In a concentration of 10 mg/kg, for instance, euphylline caused the volume velocity of the coronary blood flow to rise on the average by 45% for a period of 30 minutes.

Measurements of the oxygen demand of the heart showed that euphylline, while increasing the rate of the coronary blood flow, increased the uptake of oxygen by the heart muscle (Fig. 2). The increase in oxygen consumption by the heart usually very slightly exceeded the increase in the volume velocity of the coronary blood flow. The oxyhemoglobin concentration in the blood of the coronary sinus usually fell by 1-3% under the influence of euphylline. In some experiments the oxyhemoglobin concentration was very slightly increased (by 1-2%). The increase in the rate of the coronary blood flow under the influence of euphylline in these experiments as a rule was very sharp.

Our findings of the fall in the oxyhemoglobin concentration in the blood of the coronary sinus under the influence of euphylline are in agreement with those of Foltz et al. [7]. According to these workers, euphylline caused an even more pronounced fall in the oxyhemoglobin content of the blood of the coronary sinus.

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*These experiments were carried out by Candidate Biol. Sci. E. P. Chetverikova, to whom we extend our thanks.

Changes in the arterial pressure by the action of euphylline were insignificant. In small concentrations (1-3 mg/kg), euphylline as a rule caused the arterial pressure to rise by 5-10 mm Hg, and in large doses (5-10 mg/kg) it usually lowered the arterial pressure by 10-20 mm. However, the pressure level was quickly restored (in 2-3 minutes). We found no relationship between the changes in the rate of the coronary blood flow and the reaction of the arterial pressure to euphylline.

When the minute volume was measured, just as in the experiments with papaverine, some increase in the minute volume of the heart was observed to accompany the very slight reaction that followed immediately after injection of euphylline. In those experiments in which the initial level of the arterial pressure was low (60 mm), the depressor reaction to euphylline was accompanied by a fall in the minute volume.

The work of the heart was insignificantly affected by the action of euphylline.

Euphylline caused an increase in the heart rate. The maximum increase in the heart rate was observed 2-3 minutes after injection of the drug (5 mg/kg), and amounted to 7-15% by comparison with its initial level, and moreover the heart rate had not usually returned to its initial level at the moment when the rate of the coronary circulation was restored.

Experiments with dibazol. As we have previously shown [2], the action of dibazol on the rate of the coronary circulation is very transient. After injection of dibazol in a dose of 10 mg/kg, the volume velocity of the coronary circulation rose on the average by 40%. The increase in the rate of the blood flow, however, lasted only 2-3 minutes. The increase in the volume velocity of the coronary circulation was usually followed by a slight decrease in this velocity.

Comparison between the changes in the rate of outflow of blood from the coronary sinus under the influence of dibazol and the changes in the oxygen demand of the heart showed that these processes followed approximately the same course (Fig. 3). The curve of uptake of oxygen by the heart at first rose slightly and then fell below its initial level. So also did the curve of the change in the rate of the coronary circulation. It must be pointed out that dibazol caused a greater increase than did papaverine in the oxyhemoglobin concentration in the blood of the coronary sinus. The oxygen content of the blood of the coronary sinus rose by 7-12% of oxyhemoglobin under the influence of dibazol. In some experiments the oxyhemoglobin content was increased even more — by 15-20%. This usually occurred when the oxygen demand of the heart muscle was sharply reduced, but the outflow of blood from the coronary sinus was diminished to a far greater degree.

The fall in the arterial pressure under the influence of dibazol was of a marked character. In a dose of 3 mg/kg, dibazol caused the arterial pressure to fall by 12-20 mm for a period of 3-7 minutes, and in a dose of 10 mg/kg, by 30-40 mm for a period of action of 7-15 minutes. Just as in the experiments with papaverine, immediately after the depressor reaction, in some cases a slight increase in arterial pressure above initial level took place. The maximum increase in the rate of the coronary circulation under the influence of dibazol occurred during the period of the most marked fall in the arterial pressure.

The minute volume and the work of the left heart were diminished by the action of dibazol. Only in one of the experiments was an increase in the minute volume observed to accompany an insignificant fall in the arterial pressure.

Heart rate was decreased by from 5 to 15% after injection of dibazol. The changes in the heart rate lasted longer than the changes in the rate of the coronary blood flow.

SUMMARY

Experiments were performed on anesthetized cats. The author studied the effect of papaverine, euphylline, and dibazol on the volume velocity of the blood outflow from the coronary sinus, the oxygen uptake by the heart and the work of the heart. As shown by the experiments, the changes of arterial blood pressure, minute volume, the work of the heart, and the frequency of the heart beat do not play a decisive role in the increase of the volume velocity of the coronary blood flow under the effect of the drugs under investigation. The changes of the volume velocity of the coronary blood flow and the heart oxygen uptake under the effects of the above vasculo-dilating preparations are strikingly parallel.

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*See C. B. Translation.

THE SYNERGISM BETWEEN POLYSULFIDES AND ANESTHETICS

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Polysulfide compounds of the thiuram group (tetraethylthiuramdisulfide and tetramethylthiuramdisulfide) have found application in industry as vulcanizers in the production of various types of rubber. In recent years these compounds and their semimolecules (sodium diethyldithiocarbamate and dimethyldithiocarbamate) have begun to be widely used in agriculture in the protection of various crops from blight, molds, oscochitosis of peas and so on [2, 6, 7].

As we have previously shown, these preparations exert a well-marked action on the blood and hemopoietic organs of animals [4, 5]. One of them – tetramethylthiuramdisulfide – conventionally known as "tetrathion" has been recommended for clinical trial as a drug superior in many of its properties to embikhin, novembikhin, etc.

Antabuse (tetraethylthiuramdisulfide) has found application in medicine in the treatment of chronic alcoholism [1, 9, 10, 11, 12]. The mechanism of its therapeutic action is not yet fully understood. It is thought to be based on the principle of potentiated synergism [3, 8, 15]. By blocking the xanthine oxidase of the liver, antabuse increases and distorts the effect of alcohol [15].

We have found that the three other compounds mentioned above have the property of increasing and prolonging the action of alcohol. The problem of the combined action of these compounds with other narcotics has not been investigated at all, although such a study would be of great interest in view of the widespread use of derivatives of thiuram in agriculture, industry, and medicine.

On the basis of the few papers [13, 14] published on antabuse, it may be considered that thiuram derivatives in all probability enhance the action of other narcotics besides alcohol.

In the present research we studied the combined action of antabuse, tetrathion, and sodium diethyldithiocarbamate and dimethyldithiocarbamate with narcotics (hexobarbital, sodium amytal, chloral hydrate, and urethane).

EXPERIMENTAL METHODS

Experiments were carried out on 200 mice (mainly males) weighing from 16 to 28 g. Polysulfides were introduced into the stomach of the animals on two occasions (once a day) in the form of a 1% suspension in 2% starch mucilage, in a dose of 100 mg/kg. In this dose it gave rise to no visible changes in the mice. Before the experiment the animals were divided into groups, each of 10 mice. During the first day one group of animals received antabuse, another – tetrathion and the following groups received the other polysulfides. The control group (10 mice) received the same volume of 2% starch mucilage. On another day, 30 minutes after the second administration of these compounds, all the mice including the controls) received subcutaneous injections of narcotics in a dose sufficient to cause narcosis: hexobarbital – 35 mg/kg, sodium amytal – 80 mg/kg, chloral hydrate – 400 mg/kg and urethane – 1500 mg/kg.

TABLE

Results of a Study of the Combined Action of Polysulfides and Narcotics

Narcotic	Experiment: polysulfides: tetrathion		Na dimethyldithiocarbamate		antabuse		Na diethyldithiocarbamate		Control: 2% starch mucilage	
	1	2	1	2	1	2	1	2	1	2
Hexobarbital	70	—	38	—	31	—	24	—	26	—
Sodium amytal	709	1	752	—	732	—	807	2	164	1
Chloral hydrate	249	—	900	6	627	5	148	—	107	—
Urethane	491	10	954	7	3 314	5	1 296	2	517	1

Note: 1) Duration of LP in minutes; 2) number of dying mice.

Synergism between the test compounds and narcotics was judged by the duration of narcosis and by death of the animals. The duration of narcosis was deduced from the "lateral position" (LP); the end of narcosis was considered to be the moment when the animal began to wake spontaneously and to show its ability to move about. Careful records were kept of the results of the experiments. The mean duration of narcosis was obtained from these findings (see Table).

EXPERIMENTAL RESULTS

The experiments showed that a well-marked synergism existed between the polysulfides and narcotics. This was shown not only by the prolongation but, in some cases, by the intensification of the narcotic action of the drugs tested.

Combined action of polysulfides with hexobarbital. It may be seen from the table that tetrathion had the strongest action in prolonging the narcotic effect of hexobarbital. The duration of narcosis in the presence of tetrathion was increased from 26 minutes (control figure) to 70 minutes. Antabuse and sodium dimethyldithiocarbamate caused a very slight prolongation of not more than 5-12 minutes by comparison with the controls.

Combined action of polysulfides with sodium amytal. In this series of experiments all the preparations brought about a roughly equally marked prolongation of the narcotic action of sodium amytal. Sodium diethyldithiocarbamate lengthened narcosis 4.9 times (from 164 to 807 minutes), sodium dimethyldithiocarbamate — 4.5 times (from 164 to 752 minutes), antabuse — 4.4 times (from 164 to 732 minutes) and tetrathion — 4.3 times (from 164 to 709 minutes) by comparison with the controls.

Combined action of polysulfides with chloral hydrate. It can be seen from the table that sodium dimethyldithiocarbamate and antabuse, in prolonging the course of the narcosis, considerably enhanced the narcotic action of chloral hydrate; this was shown by the death rate among the animals of these groups by comparison with the controls. In the presence of sodium dimethyldithiocarbamate, for instance, the duration of narcosis was lengthened 8.4 times (from 107 to 900 minutes) and 6 of the 10 mice died, but not one control animal died. When combined with antabuse, narcosis was prolonged from 107 to 627 minutes (5.9 times); 5 of these mice died. In the presence of tetrathion the duration of narcosis rose from 107 to 249 minutes, and of sodium diethyldithiocarbamate — from 107 to 148 minutes.

Combined action of polysufides with urethane. In these experiments, just as in those with chloral hydrate, synergism was observed in both strength and duration of the effect. When antabuse was combined with urethane 5 mice died and one in the control group. The duration of narcosis increased from 517 minutes (controls) to 3314 minutes (by 6.4 times). With sodium diethyldithiocarbamate narcosis was prolonged 2.4 times (from 517 to 1296 minutes). In association with the action of sodium dimethyldithiocarbamate the duration of narcosis increased from 517 to 954 minutes. At the same time many of the animals were observed to die: 7 of the 10 mice taking

part in the experiment died (6 mice more than in the control series). When tetrathion was combined with urethane all 10 mice died, but only one in the control series. The period of narcosis was shortened from 517 to 491 minutes, i.e., by an average of 26 minutes.

SUMMARY

It may be concluded from these experiments that polysulfides of the thiuram group (antabuse, tetrathion, sodium dimethyl- and diethyldithiocarbamates) prolong and, in some cases also enhance the action of narcotics. Care must be exercised when prescribing sodium amytal, chloral hydrate, and urethane to alcoholics under treatment with antabuse. These narcotics must not be prescribed in cases of accidental poisoning by polysulfides of the thiuram group.

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A COMPARATIVE STUDY OF THE FIXATION OF ANTIBIOTICS OF THE TETRACYCLINE GROUP BY HOMOGENATES OF ORGANS

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The least studied aspect of the distribution of antibiotics in the body is their fixation by the organs. The volume of experimental data in this field seems not in accordance with the importance of the subject. This is to some extent due to technical difficulties.

Our aim was to obtain comparative quantitative data on the fixation of chlortetracycline, tetracycline, and oxytetracycline by organ homogenates.

In the literature there are only isolated communications on this problem. In one of these it is stated that a homogenate of guinea-pig liver fixes tetracycline to a greater degree than homogenates of other organs [4]; there are similar reports concerning oxytetracycline, but in experiments with fragments of organs rather than with homogenates [3]. In both these researches, the workers confined themselves to a comparison of the degree of fixation of the antibiotic by various organs, without examining the quantitative aspect of the subject.

EXPERIMENTAL METHODS

Experiments were carried out on the organs of white rats. In order to secure exsanguination of the animals, the axillary artery and vein were divided under light ether anesthesia; the liver, kidneys, spleen, heart, lungs, and brain were then extracted from the animals and equal samples by weight of these organs were homogenized with a measured quantity of quartz sand. The homogenate was transferred into a centrifuge tube, where to it was added a solution of the antibiotic in phosphate buffer (pH = 7.4) in a dose calculated as 0.3 ml of a concentration of 20 γ/ml per 0.7 g of tissue. Thus 1 g of the mixture (antibiotic solution and organ homogenate) contained 6 γ of chlortetracycline, tetracycline, or oxytetracycline. The proportion of 0.3 ml to 0.7 g was adopted because in preliminary experiments it was found that the degree of fixation fell as the volume of antibiotic solution increased in relation to the weight of homogenate. A smaller volume of antibiotic solution was not used, for this made the determination of the activity of the mince much more difficult, on account of its viscosity. The contents of the centrifuge tube were agitated on a shaker (280 agitations per minute) for 30 minutes and were then centrifuged at 3000 rpm, also for 30 minutes. Subsequently the activity of the supernatant fluid was determined on plates seeded with the spore-bearing strain L_2. The standard was also dissolved in phosphate buffer. Control experiments showed that none of the homogenates of the organs used in the experiment formed zones of inhibition of growth on the plates. From 5 to 14 experiments were performed with each organ and each antibiotic.

TABLE

Fall in the Activity of the Tetracyclines in the Presence of Homogenates of the Organs of Rats (Estimated Activity 6 γ/ml)•

Organs	Antibiotics								
	chlortetracycline			tetracycline			oxytetracycline		
	activity (in γ/ml)•	m	fixation (in %)	activity in γ/ml•	m	fixation (in %)	activity in γ/ml•	m	fixation (in %)
Liver	2.3	0.18	61.6	2.9	0.19	51.6	3.8	0.28	38.3
Kidneys	2.5	0.12	58.3	3.1	0.42	48.3	4.5	0.44	25.0
Spleen	4.6	0.52	23.3	—	—	—••	—	—	—••
Lungs	3.9	0.37	35.0	4.4	0.55	26.6	—	—	—••
Heart	5.4	0.64	10.0	5.1	0.83	15.0	—	—	—••
Brain	2.3	0.19	61.6	3.8	0.21	38.3	5.6	0.56	6.6

• Results of the determination of activity were treated statistically; possible error in the value of $M \pm m$ does not exceed 0.1% (by Pomorskii's method).

•• No fixation discovered.

EXPERIMENTAL RESULTS

It was found that the organ homogenates fixed different quantities of the tetracyclines; here, as in the serum [2], chlortetracycline was fixed to the greatest degree, closely followed by tetracycline, and oxytetracycline was fixed to the smallest degree (see Table).

By the degree of fixation of the tetracyclines, the organ homogenates could be divided into 2 groups: those fixing considerable amounts of tetracyclines – the liver, kidneys, and brain, and those fixing appreciably smaller amounts – the lungs, heart, and spleen. In all cases the liver fixed the largest amount of tetracyclines (from 61.6% in the case of chlortetracycline to 38.3% in that of oxytetracycline).

The results obtained with homogenate of the brain were unexpected: It fixed chlortetracycline to the same degree as homogenate of liver and tetracycline to a less but still considerable degree (38.3%). Fixation of oxytetracycline by homogenate of brain was slight (6.6%), but it must be remembered that homogenates of the lungs, heart, and spleen did not in general fix this antibiotic in our experiments.

In earlier experiments in which rats were given tetracyclines by mouth, and their organs were subsequently homogenized and repeatedly rinsed, we found no antibiotics in the brain tissue [1]. The results of the present research suggest that this is explained by the low permeability of the blood-brain barrier to the tetracyclines, since when these antibiotics are brought into direct contact with brain tissue they undergo absorption by it in large quantitites.

The problem of the reversibility of the bond between the tetracyclines and the brain homogenate remained unsolved. In previous work we were unable to explain this for, as has been stated, these antibiotics were not found in the brain tissue. In the present work, by repeated washing of chlortetracycline from the brain homogenate roughly the same amount of the antibiotic could be extracted from it as from liver homogenate, used as a control. The bond between brain, like the other organs, and the tetracyclines is thus reversible.

From these findings and the results of our previous work [1, 2], we can begin to understand some of the factors governing the distribution of the tetracyclines in the body. The low fixation of oxytetracycline by the serum and organs may account for its high concentration in the blood when injected intravenously. Chlortetracycline and tetracycline do not give such high blood levels, but because of the considerable deposition of the antibiotic in the organs and serum, it remains in the body far longer.

From the point of view of rational chemotherapy it may be supposed from our findings that chlortetracycline and tetracycline will be more effective in infectious diseases localized in the organs, and especially in the liver and kidneys. In generalized septicemic conditions ocytetracycline may be of essential importance. In these cases it may be rational to combine it with chlortetracycline or tetracycline.

SUMMARY

The authors established the quantitative fixation of chlortetracycline, tetracycline, and oxytetracycline by homogenates of the liver, kidneys, spleen, lungs, heart, and brain of white rats. Fixation by the organ homogenates was greatest by chlortetracycline, followed by tetracycline and oxytetracycline in that order. Homogenates of the liver, kidneys, and brain fixed the tetracyclines in considerable quantitities, those of the lungs, heart and spleen – to a noticeably lesser degree.

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THE REFLEX EFFECT OF CERTAIN DRUGS ACTING ON THE RECEPTORS OF THE MESENTERIC VEINS IN THE CAT

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The presence of receptors in the veins, reacting to various chemical stimuli, has been demonstrated by a number of investigations [1, 2, 5, 7, 8, 9, 10, 11]. In our previous research [3, 4] we showed that adrenalin, strophanthin, and certain other drugs, acting on the receptors of the lateral thoracic vein of the rabbit and the subcutaneous femoral vein of the cat, bring about a slowing of the heart rate, a fall in the arterial pressure, and depression of respiration, but when acting on the receptors of the femoral vein in the frog they depress respiration and elicit a generalized motor reaction but do not change the rate of the heart.

The present work is a continuation of the above-mentioned research.

EXPERIMENTAL METHOD

Experiments were carried out on cats in order to study the reflex effects of drugs acting on the receptors of the mesenteric vein, using the method of perfusion of a humorally isolated segment of vein. The humoral isolation of an area of the mesenteric vein was carried out under urethane anesthesia by the method suggested by B. Z. Sirotin [6]. After the animal had been fixed to the bench, a 10% solution of urethane was injected intraperitoneally in a dose of 1-2 g/kg body weight. The animal was subsequently maintained in a state of light anesthesia by injections of small doses of the anesthetic.

Ringer's solution or the test solutions were passed through the humorally isolated segment of the mesenteric vein, 8-12 cm in length. The level of the solutions was maintained 10-12 cm above the perfused segment of vein and was unchanged throughout the experiment.

As a result of the free drainage, the pressure in the isolated segment of vein did not exceed the blood pressure in the other veins of the peritoneal cavity of the animal, as was shown by the entry of blood into the perfusion fluid from small divided vessels. The aerated solutions (test and Ringer's) were warmed to 37-38° before being introduced into the segment of the vein. After the perfusion had begun, the intestine was replaced in the peritoneal cavity, where it remained throughout the experiment.

Changes in the activity of the heart were recorded by an EKP-4 electrocardiograph. The action potentials of the heart were picked up by means of needle electrodes introduced beneath the skin corresponding to lead 2. The electrocardiogram was recorded several times before application of the stimulus, during stimulation, and after the chemicals had been rinsed out with Ringer's solution. Continuous kymographic tracings of the arterial pressure in the carotid artery were made with a mercury manometer and of the respiration through a tracheal cannula.

In each experiment from 2 to 7 tests were made of the reflex effect of the test substances (the same or different). When different substances were tested, the order of their application in the experiments was changed.

TABLE

Reflex Changes in the Heart Rate, Arterial Pressure, and Respiration in Response to Stimulation of the Receptors of the Mesenteric Vein of the Cat

Drug tested	Heart rate			Arterial pressure			Respiration		
	slowing	no change	quicken.	decrease	no change	increase	de-pression	no change	excitation
Adrenalin	6	2	4	–	10	8	–	11	9
Strophanthin	10	3	8	8	10	2	7	15	2
Caffeine	12	3	4	12	12	1	7	12	2
Glyceryl	4	1	8	6	10	–	5	10	–
Cardiazol	4	1	2	7	6	1	8	3	2
Total number of tests	70			93			93		

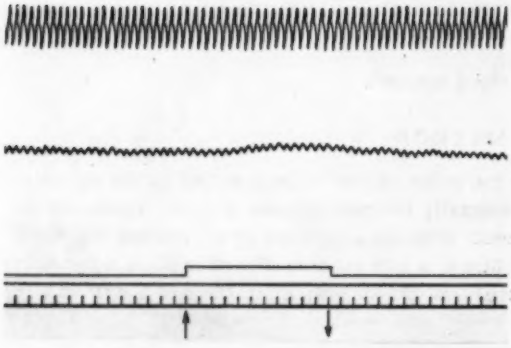

Fig. 1. The reflex effect of a $1 : 10^6$ adrenalin solution from the receptors of the mesenteric vein of a cat on the respiration and arterial pressure. Significance of the curves (from above down): respiration, arterial pressure, stimulus marker, zero line, time marker (10 seconds). The arrows indicate the time of perfusion of the drug and of rinsing it out.

As stimuli of the receptors of the mesenteric vein we used drugs having a marked action on the cardiovascular and respiratory systems when administered to the animal. The reflex effect of the following drugs was studied: adrenalin hydrochloride in a dilution of $1 : 10^6$, caffeine sodium benzoate – $1 : 10^3$, strophanthin – $1 : 2 \cdot 10^6$, glyceryl trinitrate – $1 : 2 \cdot 10^5$ and cardiazol – $1 : 2 \cdot 10^6$.

EXPERIMENTAL RESULTS

The method of perfusion of the humorally isolated mesenteric vein was used in investigations on 20 cats. The results of the experiments are shown in the table.

Adrenalin, strophanthin, glyceryl trinitrate, and cardiazol, in roughly the same number of tests, caused either a reduction in the heart rate or an increase. A more definite result was given by caffeine which slowed the heart rate in 12 tests, quickened it in 4, and left it unchanged in 3 tests. The reflex changes in the activity of the heart by the action of the test drugs on the receptors of the mesenteric vein were insignificant in our experiments and did not exceed 10-15 beats per minute. In only one experiment did caffeine increase the heart rate by 25 beats. A second test of caffeine in the same experiment, instead of increasing the heart rate, decreased it by 7 beats. We observed this inconstancy of reflex action on the heart rate in many experiments in which the effect of other drugs besides caffeine was tested.

Thus in experiments on the mesenteric vein of the cat, as in those on the lateral thoracic vein of the rabbit and the subcutaneous femoral vein of the cat [3], results were obtained which show the absence of any specific, strictly purposeful reflex effect from the receptors of the veins on the activity of the heart.

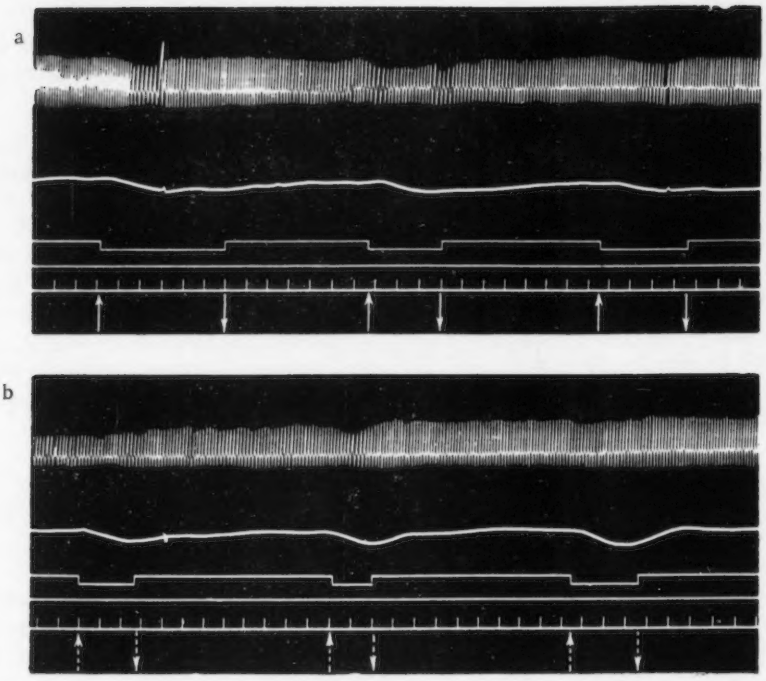

Fig. 2. Reflex effect from the receptors of the mesenteric vein on the respiration and arterial pressure of a cat.
a) Injection of $1:2\cdot10^{6}$ cardiazol solution; b) injection of $1:10^{3}$ caffeine solution. Significance of the curves as in Fig. 1.

Qualitative changes in the electrocardiogram were studied from the results of 18 experiments. We could not detect any characteristic changes connected with the action of any of these drugs on the receptors of the mesenteric vein. During quickening and slowing of the heart rate the P wave was diminished, but the R and T waves and the S — T interval showed inconstant changes.

The direction of the reflex changes in the arterial pressure and respiration are shown in the table. The most obvious effect from the receptors of the mesenteric vein on the arterial pressure and respiration was shown by adrenalin. In almost one half of the tests it caused an increase in the arterial pressure by 5-10 mm Hg and strengthening of respiration. Adrenalin did not lower the arterial pressure in any experiment, nor did it depress respiration. In the majority of effective tests of the action of strophanthin, caffeine, glyceryl trinitrate, and cardiazol on the receptors of the mesenteric vein the arterial pressure was lowered by 5-30 mm Hg and the respiration was depressed.

By the action of the test drugs on the receptors of the mesenteric vein, changes developed gradually in the arterial pressure, which then returned smoothly to its initial level (Figs. 1, 2). In some experiments, after the first wave of fall or rise of arterial pressure, coinciding in time with the action of the stimulus on the receptors, a second fall or rise of arterial pressure ensued while the vein was being rinsed with Ringer's solution.

In the majority of tests the fall in arterial pressure was accompanied by depression of respiration, or sometimes by excitation. In some experiments changes occurred in the arterial pressure while respiration remained unchanged (see Fig. 1). In other experiments obvious changes developed in the respiration although the arterial pressure remained constant. The changes in respiration and arterial pressure did not always coincide in duration.

After the repeated use of the same drug as a stimulus of the receptors of the mesenteric vein, a gradual diminution of the reflex response was observed, in some cases as far as absence of reaction to this stimulus. Perfusion of the isolated segment of vein with another drug, however, caused reflex reactions for a further few tests. Sometimes, in response to the repeated testing of the same drug, the first test gave no reaction, the second gave a reaction in one direction and the third, a reaction in the opposite direction.

Changes in respiration in response to the action of the drugs on the receptors of the mesenteric vein usually developed gradually and recovered smoothly. In most experiments simultaneous changes were observed in the depth and rate of respiration (see Fig. 2), but in some cases reactions affecting either the depth or the rate of respiration were seen.

The facts and experimental findings described show that perfusion of the humorally isolated segment of the mesenteric vein of the cat with the drugs that were studied may give rise to obvious changes in the arterial pressure and respiration. In order to prove their reflex nature, 5 control experiments were carried out in which the function of the receptors was blocked with 2% procaine, and 2 experiments in which labeled phosphorus P^{32} was used for this purpose. When the isolated segment of vein was perfused with the drugs after perfusion with 2% procaine solution for 10 minutes, no reflex changes were produced in the arterial pressure or respiration. In samples of blood taken from the subcutaneous femoral vein of the cat after perfusion of the isolated segment of mesenteric vein with the phosphorus isotope, the number of impulses per minute showed no increase after 15, 30, 60, and 120 minutes. The method which we adopted gave assurance of the humoral isolation of the isolated segment of vein, while preserving its nervous connections with the rest of the body intact, so that the reactions observed were reflex in nature.

The results of these experiments suggest that the reflexogenic zone of the intestinal veins plays a part in the mechanism of action of drugs entering these veins after absorption from the intestine.

SUMMARY

By employing the perfusion method of the humorally isolated portion of the anterior mesenteric vein in cat the author studied the reflex effect of solutions of adrenalin ($1:10^6$), caffeine sodium benzoate ($1:10^3$), strophanthin ($1:2\cdot10^6$), glyceryl trinitrate ($1:2\cdot10^5$) and cardiazol ($1:2\cdot10^6$) on the heart rate, arterial pressure, and respiration. Adrenalin provoked a rise of arterial pressure and stimulation of respiration by reflex action, while other substances mostly reduced the arterial pressure and depressed the respiration. Caffeine frequently decreased the rate heart. The reflex origin of these reactions was proved by control experiments with 2% procaine solution and labeled phosphorus P^{32}.

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THE GROWTH AND DEVELOPMENT OF BACTERIA IN CONNECTION WITH THE PHENOMENON OF BACTERIOPHAGE

COMMUNICATION VII. FURTHER INVESTIGATIONS OF THE NUCLEAR STRUCTURES OF BACTERIA AND OF THE INFLUENCE OF PHAGE THEREON

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In a previous communication we gave experimental findings relating to the nuclear structures of cells of Escherichia coli aerogenes, studied by means of the electron microscope and microchemical analysis, and we put forward certain views on the general structure of the nuclear apparatus of bacteria [3].

In the present communication we describe the results of further submicroscopic investigations of the finer points of the nuclear structures and of their connection with the division of bacteria, and we also give data on the effect of phage on these structures.

EXPERIMENTAL METHODS

The investigations were conducted on ordinary and ultrafine serial sections of cells of 4-hour cultures of E. coli B., grown under aerobic conditions, and also on sections of cells of the same cultures of E. coli B, but infected with T2 phage to an infection coefficient of 1:1.3. The preparations were made 5 minutes and 2 hours after the beginning of infection, which was interrupted by cooling of the cultures. In selecting these time intervals after infecting the bacteria with phage, we were profiting from previous experience [2] that the study of lysis due to bacteriophage after successive intervals of time presents no advantages when the trend of the changes is being determined, for not all cells react in the same way to infection with phage in the course of time. The widest range of morphological changes in the E. coli cells is observed 1½–2 hours after the beginning of infection, when different morphological forms preceding lysis may be found [2].

In preparing the sections the fixation and embedding of the bacteria in methacrylate were done according to a scheme which differed slightly from that used in the previous work [3], namely:

room temperature,	0.1% OsO_4 (pH = 7.2–7.4)	– 30 minutes (not changed)
" "	0.7% OsO_4 (pH = 7.2–7.4)	– 2 hours, " "
cold.	70% alcohol	– overnight " "
room temperature,	95% alcohol	– 2 hours " "
" "	absolute alcohol	– 2 hours " "
" "	mixture of methacrylates, catalyst, and alcohol (absolute) in eq. vols.	–30 minutes (not changed)

Fig. 1. Longitudinal and transverse sections of E. coli B cells with nuclear structures. Magnification 123,000.

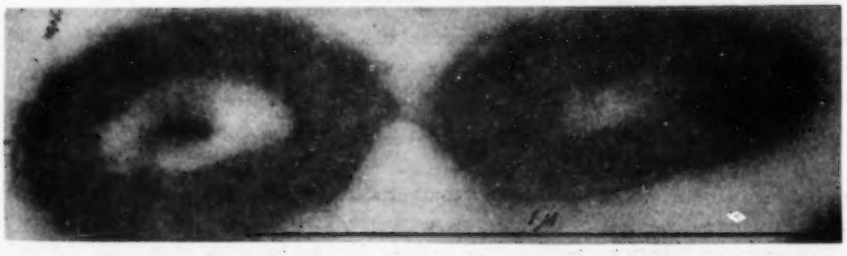

Fig. 2. Section through an almost completely divided E. coli B cell. Magnification 123,000.

room temperature, mixture of methacrylates and catalyst – 3 hours (changed every hour)
48–48°. . . . . mixture of methacrylates and catalyst – 48 hours (in capsules).

Besides methacrylates, araldite was used for embedding of some specimens after dehydration [7] according to the following scheme:

room temperature, mixture of araldite mixture and absolute alcohol in equal volumes – 30 minutes (not changed).
48°, araldite mixture – 3 hours (changed every hour)
48°, " " – 1 hour (changed every 30 minutes)
48°, araldite mixture with catalyst – 1½ hours (changed every 30 minutes)
48°, araldite mixture with catalyst – 30 hours (in capsules).

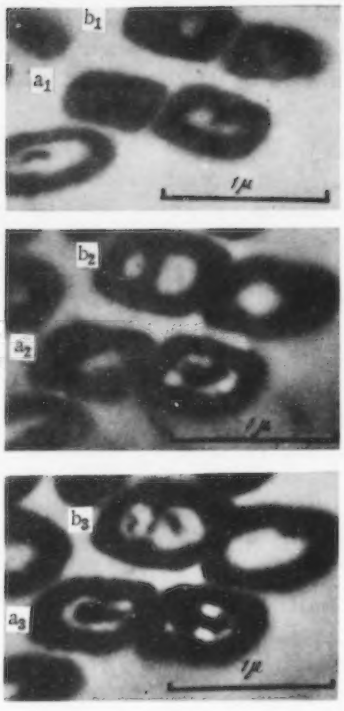

Fig. 3. Serial sections of E. coli B.cells in process of division (a, b). Magnification 36,000.

We thus had at the same time specimens embedded in methacrylates and araldite after the same methods of fixation and dehydration. The order of fixation and embedding adopted allowed the structures to be preserved and enabled sections to be obtained which were suitable for high resolution electron microscopy.

Serial sections were obtained by means of a Sjostrand ultramicrotome. The knives were of glass. Moderate and high resolution electron microscopy was undertaken.

Fig. 4. Sections of E. coli B.cells and their nuclear structures, modified by the action of phage. Magnification 81,700.

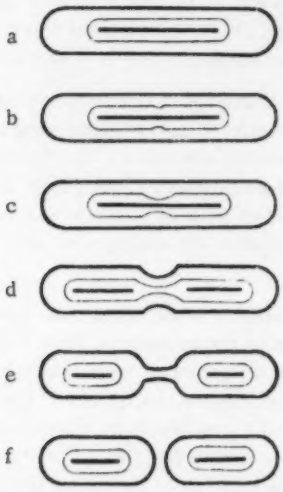

Fig. 5. Scheme of division of a bacterial nucleus. a) Resting cell; b) beginning of division of the nucleus; c) continuation of division of the nucleus; d) division of the chromatin band and beginning of indrawing of the membrane; e) end of division of the nucleus; f) division of the cell completed.

EXPERIMENTAL RESULTS

As in the experiments with ultrathin sections of E. coli aerogenes, we obtained photographs of ultrathin sections of cells of E. coli B., in which we observed the same peculiarities in the structures of the nuclear formations. Different methods of embedding gave the same results from this point of view.

Fig. 1 shows longitudinal and transverse ultrathin sections of E. coli B.cells. Despite the fact that the observations were made at high resolution of the microscope, we were unable to demonstrate the presence of membranes enveloping the nuclear formations in these sections, whereas at this resolution the double cell membranes showed up very well. The borders between the fine granular cytoplasm and the nuclear contents showed up very clearly, however, on all the films. This enabled some idea to be obtained of the area occupied by the nuclear structures in relation to the total area of the bacterial cell.

The study of many photographs of sections showed that the dimensions of the dense, longitudinal chromatin bands were not the same in all the cells. With a thickness on cross section of roughly 120 to 720 A, in individual sections these bands reached 5000 A and more in length. In none of the sections were these bands observed to begin directly from the borders with the cytoplasm; usually their ends were situated at a short distance from this border. Attention was drawn to the structure of the chromatin bands. On the films they had the character of continuous structures with no signs of splitting up into smaller elements.

The character of the nuclear formations in the dividing bacterial cells could also be examined in the sections. In the cells in which the process of division was very clearly expressed, as may be seen, for example, in Figs. 1, 2, and 3 (serial sections a_1, a_2 and a_3), nuclear substance was present in the form of light zones in which were included dense bands one by one in each part of the dividing or almost completely divided cells, in which only a small band was left of the cell membrane. It could be seen from the serial sections, on the other hand, that the process of division of the nuclear formations begins before signs of division are seen in the cell membrane and cytoplasm. Moreover, division of the nuclear formations, as can readily be seen in Fig. 3 (serial sections b_1, b_2 and b_3) took place in the newly formed cells at a time when the process of division of the original cell was not yet complete. These observations thus show that division of nuclear structures begins and ends sooner than the division of the bacterial cell as a whole to which they belong.

Electron microscopy of ultrathin sections of E. coli B cells infected with T2 phage, 5 minutes after the beginning of infection, i.e., 5 minutes after the addition of phage particles to the bacteria, revealed no appreciable changes in the nuclear apparatus of the bacteria. Such changes were readily demonstrated in preparations of bacteria which were prepared 2 hours after the beginning of infection. In a field of vision of such preparations were seen sections of cells of different shapes, beginning with some in which the changes in the external shape were hardly noticeable and ending with others which were converted into oval and spherical forms, usually preceding lysis.

The first thing to be observed from a study of the photographs of the sections was that the character of the nuclear structures in the sections of those cells that were unchanged or only slightly changed showed essential differences from the character of these structures in the sections of the oval and spherical cells. In the sections of unchanged or only slightly changed cells the light zones of nuclear substance were completely intact, although newly formed phage particles were to be found there. In shape they corresponded to the nuclear zone of cells not subjected to infection (Fig. 4). The chromatin bands were also preserved, although on account of a decrease in their density in relation to electrons, they were difficult to make out at this moment against the background of the cytoplasm.

In the cells in which the process of change in the external form was clearly expressed, light zones in the form of well defined central areas were absent; in the sections they had the appearance of small areas of ill-defined shape, scattered over the whole surface (see Fig. 4). It was characteristic that there were far fewer of these areas in the sections of the spherical cells, and their borders were more indistinct. Chromatin bands, as observed in the light zones of the nuclear substance of normal bacteria, were not found here. Thus, if the morphological changes in bacteria preceding lysis were far advanced, almost complete disintegration of the nuclear material was observed.

In selecting the test object for the present investigation, our aim was to choose a strain of E. coli which would have been most thoroughly studied in this direction, so that comparable results would be available for the formation of conclusions and for their discussion. We considered that E. coli strain B answered this purpose.

Our findings in relation to the structure of the nuclear apparatus of E. coli B were in complete agreement with the results of our previous experiments [3] in which observations were made on the nuclear apparatus of E. coli aerogenes, and with the conclusions drawn therefrom, and also with the data on the nuclear structures of E. coli B, published abroad [8, 10], where this strain of E. coli has been the subject of extensive cytomorphological study in recent years.

The use of serial ultrathin sections has revealed new and finer details of the structure of the nuclear apparatus and given additional evidence in support of the views which we have developed: In our opinion the nuclear apparatus of bacteria consists of distinctive vacuoles situated in the central part of the internal contents of the cells, and filled with material readily permeable to electrons, containing dense chromatin bands which are elongated in a longitudinal direction [3].

Like other authors [5, 9], we were unable to detect a membrane of the nuclear apparatus, although its absence is not yet finally settled. The presence of such a well-defined border between the nuclear structures and the cytoplasm, and also the possible use of a microscope with a higher resolving power (we worked with resolution of the order of 15 A) provide a basis for further research in this direction.

The data described above on the division of the nuclear apparatus of bacteria undoubtedly indicate the leading role of this process in the division of the bacterial cell as a whole, just as takes place in the division of higher forms of cells. From an analysis of the morphological pictures of the dividing nuclei in serial ultrathin sections of bacteria it may be concluded that the division of the nuclear structures, which initiates the division of the bacterial cell, corresponds in general outline to the scheme illustrated in Fig. 5. It can readily be seen that this scheme may be defined as amitosis. This conclusion disagrees with that of De Lamater [6], who considers, on the basis of optical and microchemical observations, that the nucleus of bacteria divides by karyokinesis.

The electron microscope pictures of the changes in the nuclear apparatus of bacteria under the influence of phage, which are described here, fully agree with the microchemical findings reported in the literature on this subject [1, 4, 11]. In conjuntion with the latter, our material shows that during infection of bacteria with phage, the destructive changes begin in the nuclear structures. Discrete nuclear structures are no longer visible in the changed bacterial cells before undergoing lysis.

The results of our experiments thus demonstrate the leading role of the nuclear structures both in processes connected with division of the bacterial cell and in the destructive changes in the bacterial cell resulting from phage infection.

In conclusion the author expresses his gratitude to Professor Sjostrand for his technical advice in the performance of this research, and also to laboratory assistant Cayland for technical assistance (from the Laboratory of Biological Ultra-structures, Karolinska Institute, Stockholm, Sweden).

SUMMARY

Electron microscopy of serial ultrathin sections of E. coli B cells revealed division of bacterial nuclear structures which may be regarded as being amitotic in character. Destructive changes which take place in phage infection of bacteria commence in the nuclear structures. Data on the nuclear apparatus and the effect of phage thereon demonstrate the leading role of the nuclear structures not only in processes connected with multiplication, but also in cases of destructive changes occuring in bacterial cells as the result of phage infection.

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*See C. B. Translation.

INVESTIGATION OF THE "VALENCY" OF ANTIBODIES BY MEANS OF AZOPROTEINS

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In a previous communication [1] we showed that a mixture of monoazo derivatives, and binary azo derivatives of proteins give rise to the same effect when used as test antigens in immunization and in the ring precipitation test. Under these circumstances complete suppression of the specificity of one hapten by the other was not observed i.e., antibodies to both haptens contained in the antigen were found in the immune sera in all cases. It is of considerable interest to determine the number of specifically reacting groups in these antigens and thereby to pave the way for the investigation of the problem of the "valency" of antibodies.

No unanimity exists in the immunochemical literature on this subject [3, 4, 6-12, 14, 15].

In order to study this problem we used the method of exhaustion of antisera against azoproteins, followed by performance of the cross precipitation reaction both with antibodies remaining in the supernatant portion of the serum and with the antibodies contained in the precipitate, after preliminary separation from antigen.

EXPERIMENTAL METHODS

We obtained mono- and diazo derivatives and used them to immunize rabbits as in the previous work [1]. We investigated an immune serum to horse sulfanylanthranylazoglobulin and an immune serum to a mixture of horse sulfanylazoglobulin and human anthranylazoglobulin. To determine the strength of the serum (the highest dilution of the test antigen) and to ensure complete precipitation of the antibodies (maximum precipitate with the test antigen) the precipitation reaction was performed in a volume of 1 ml (0.5 ml of antigen and 0.5 ml of immune serum). The test antigens were standardized by weight; an 8% stock solution was used and from it were prepared dilutions of 1:2, 1:4, 1:16 and so on.

To exhaust the antibodies, 10 ml of antiserum was mixed with 10 ml of antigen in the appropriate dilution. The mixture was allowed to stand for 2 hours in the incubator and then left at room temperature overnight. The liquid was separated from the precipitate by centrifugation. Completeness of precipitation of the antibodies was tested by the addition of a portion of antigen to 0.5 ml of the centrifugate. In the absence of a precipitate the exhaustion of the serum was considered complete.

EXPERIMENTAL RESULTS

The change in the titer of the precipitating serum after its exhaustion is shown in Table 1.

We can see that the antiserum against a mixture of horse sulfanylazoglobulin and human anthranylazoglobulin (experiments 1-9) precipitated horse globulin, human globulin and their sulfanyl- and anthranylazo derivatives (experiment 1). In this antiserum were found antibodies whose specificity clearly and fully reflected the structure of the immunizing azoproteins. This can be seen from the fact that human sulfanylazoglobulin in

TABLE 1

Change in the Titer of a Precipitating Serum after its Exhaustion

Antiserum	Experiment No.	Antigen used for exhaustion of the serum	Maximum dilution of test antigen							
			horse globulin	horse azoglobulins			human globulin	human azoglobins		
				sulfanylazoglobulin	anthranylazoglobulin	sulfanylanthranylazoglobulin		sulfanylazoglobulin	anthranylazoglobulin	sulfanylanthranylazoglobulin
Against horse sulfanylazoglubulin + human anthranylazoblobulin	1	Unexhausted serum	16	256	128	—	64	128	256	—
	2	Horse globulin	0	128	64	—	64	128	256	—
	3	Horse sulfanylazoglobulin	0	0	64	—	64	0	256	—
	4	Horse anthranylazoglobulin	0	128	0	—	32	64	64	—
	5	Horse anthranylazoglobulin + human globulin	0	128	0	—	0	64	2	—
	6	Human globulin	16	256	128	—	0	64	128	—
	7	Human sulfanylazoglobulin	8	64	64	—	0	0	64	—
	8	Human anthranylazoglobulin	16	256	32	—	0	32	0	—
	9	Human sulfanylazoglobulin + horse globulin	0	4	32	—	0	0	64	—
Against horse sulfanylanthranylazoglobulin	10	Unexhausted serum	32	512	512	1 024	0	256	128	128
	11	Horse globulin	0	256	256	512	0	128	128	64
	12	Horse sulfanylazoglubulin	0	0	64	256	0	0	16	64
	13	Horse anthranylazoglobulin	0	128	0	256	0	32	0	32
	14	Horse sulfanyl- + anthranylazo glubulin	0	0	0	16	0	0	0	2
	15	Horse sulfanylanthranylazoglobulin	0	0	0	0	0	0	0	0
	16	Human sulfanylazoglobulin	4	64	128	512	0	0	64	32
	17	Human anthranylazoglobulin	4	256	64	512	0	128	0	64
	18	Human sulfanylanthranylazoglobulin	2	32	16	64	0	0	0	0
	19	Horse globulin + human sulfanylanthranylazoglobulin	0	0	0	4	0	0	0	0
	20	Control — normal horse serum	0	0	0	0	0	0	0	0

a solution of horse globulin did not completely extract the antibodies to horse sulfanylazoglobulin (experiment 9), just as exhaustion of the horse anthranylazoglobulin serum in a solution of human globulin did not lead to complete exhaustion of antibodies to human anthranylazoglobulin (experiment No. 5). This fact indicates that the immunological specificity of the azoproteins is determined not by the passive presence of the chemical radical combined with the protein in a certain manner, but by a complex of the chemical compound with the protein. Under the influence of chemical treatment and of the presence of a chemical radical, the protein changes its original structure and its immunological specificity.

TABLE 2

Cross Reaction with Antibodies against Horse Sulfanylanthranylazoglobulin Isolated from Precipitates

Type of antibody	Experiment No.	Antigen used for precipitation of antibodies	Test antigens: horse globulin	horse azoglobulins: sulfanyl-azo-globulin	horse azoglobulins: anthra-nylazo-globulin	horse azoglobulins: sulfanyl-anthranyl-azoglobulin	human globulin	human azoglobulins: sulfanyl-azo-globulin	human azoglobulins: anthra-nylazo-globulin	human azoglobulins: sulfanyl-anthranyl-azoglobulin
"Nonprecipitating"	1	Horse globulin	+	++	++	+++	0	++	+	++
	2	Horse sulfanylazoglobulin	±	±	++	++	0	0	±	+
	3	Horse anthranylazoglobulin	±	++	+	+++	0	++	0	++
	4	Horse sulfanylanthranylazoglobulin	0	0	0	±	0	0	0	0
	5	Human sulfanylazoglobulin	+	++	+++	+++	0	±	+	++
	6	Human anthranylazoglobulin	+	++	+	++	0	++	+	++
	7	Human sulfanylanthranylazoglobulin	±	+	+	++	0	±	±	+
"Precipitating"	8	Horse globulin	++++	+++	++	++	0	+	+	+
	9	Horse sulfanylazoglobulin	++	++++	+++	+++	0	+++	++	+++
	10	Horse anthranylazoglobulin	++	+++	+++	+++	0	++	+++	++
	11	Horse sulfanylanthranylazoglobulin	+++	+++	++	++++	0	++	++	++++
	12	Human sulfanylazoglobulin	+	+++	+	++	0	++++	++	+++
	13	Human anthranylazoglobulin	±	++	++++	+++	0	++	++++	+++
	14	Human sulfanylanthranylazoglobulin	±	++	++++	+++	0	++	++++	+++
		Control — normal horse serum	0	0	0	0	0	0	0	0

During the exhaustion of an antiserum with antigens in which the azo components were homologous but the globulins heterologous in relation to the immunizing antigen (experiments 4 and 7), a fall in the titer of antibodies to all the test antigens was observed. These findings suggest the "valency" of the antibodies to monoazoproteins. In fact, if the test antiserum contained mutually independent antibodies to each determinant, the amount of one would not be altered by exhaustion of the others. In experiment 4, horse anthranylazoglobulin (immunizing antigen – human anthranylazoglobulin) completely extracted the antibodies to horse globulin and anthranylazoglobulin and also antibodies not only to human anthranylazoglobulin but also to human sulfanylazoglobulin and globulin. Similar results were observed in experiment 7 during exhaustion of antiserum with horse sulfanylazoglobulin immunizing antigen – human sulfanylazoglobulin.

It did not seem possible to explain this extraction of antibodies by means of nonspecific adsorption, for homologous antigens (experiments 2, 3, 6, and 8) extracted only their specific antibodies. Extraction of a serum with horse sulfanylazoglobulin, for example (experiment 3) did not affect the content of antibodies to human globulin and anthranylazoglobulin.

The results of these experiments thus suggested that a test antiserum against monoazoproteins contained antibodies whose specificity was directed at the same time towards the protein and the determinant group.

Antiserum against horse sulfanylanthranylazoglobulin (experiment 10–19) precipitated horse globulin and also horse and human sulfanyl- and anthranylazoglobulin (experiment 10).

During exhaustion of a serum with horse globulin azo derivatives, complete removal of antibodies precipitating the corresponding human azoglobulins was observed (experiment 12, 13, and 15).

Complete removal of antibodies could only be achieved by the immunizing antigen – horse sulfanylanthranylazoglobulin (experiment 15), and not by mixtures of the corresponding antigens (sulfanyl + horse anthranylazoglobulin –experiment 14 – and horse globulin + human sulfanylanthranylazoglobulin – experiment 19).

In every case in this series of experiments it was found that the antigen used for exhaustion of the serum completely extracted its own antibodies and considerably reduced their titer in relation to other unrelated antigens (experiments 12, 13, 16, 17, and 18). For example, human sulfanylazoglobulin (experiment 16) exhausted not only its own antibodies but also severely reduced the titer of the antiserum to horse globulin, horse anthranylazoglobulin, and human anthranylazoglobulin. From this observation it can be concluded that a single antibody possesses simultaneous specificity to horse globulin and to human sulfanyl- and anthranylazoglobulin.

Our subsequent experiments had the object of determining the valency of the "nonprecipitating" antibodies and the antibodies present in the specific precipitates obtained by exhaustion of sera to horse sulfanylanthranylazoglobulin.

We know that "nonprecipitating" antibodies are capable of combining with antigens, but they do not form precipitates [8, 13]. In order to obtain a precipitate we used V. A. Engelgardt's method [2]. To 5 ml of serum, completely exhausted with test antigen was added 5 ml of a 1% solution of the same antigen. The mixture was thoroughly mixed and kept overnight at room temperature. After standing, the antigen – antibody complex was precipitated by the careful addition of a saturated solution of $(NH_4)_2SO_4$ until a slight opalescence appeared. After 3–4 hours a floccular precipitate was formed, which was removed by centrifugation; this was used for the isolation and study of the "nonprecipitating" antibodies. As a control we used the same serum exhausted with heterologous azoantigens. The precipitate was considered to be specific if it was formed by a concentration of the salt which did not cause precipitation in the controls.

The antibodies were isolated from the precipitates by the method of Haurowitz and his coworkers [5], with a 5% solution of NaCl in a 0.1N solution of HCl. This solution caused dissociation of the precipitate and precipitates the azoproteins. This method is applicable only to an antibody – azoantigen complex, and so for the isolation of "nonprecipitating" antibodies to horse globulin we used one of its azo compounds which was nonspecific to the test antiserum – nitroaniline-azoglobulin. Before dissociation, the precipitates were preliminarily freed from nonspecific proteins by being twice washed with a 1% solution of NaCl. In every case the antibodies were extracted by the addition of 0.5 ml of 5% NaCl solution in 0.1 N HCl to the precipitate. The mixture was thoroughly homogenized and allowed to stand at room temperature for 2 hours. The supernatant fluid was then separated by centrifugation, neutralized to pH = 7 and diluted 1:5 in order to lower the salt concentration, after which it was used in the cross precipitation reaction with the test antigens. For this purpose the fluid thus obtained and the

test antigen, diluted 1:10, were introduced drop by drop into the well of a glass slide. The well was hermetically sealed with a cover slip. After the mixture had been allowed to stand overnight at room temperature, the intensity of the precipitation reaction was observed by means of a hand lens: O – no precipitate, ± – traces of precipitate and +, ++, +++ – increasing amounts of precipitate. The experimental results are shown in Table 2.

"Nonprecipitating" antibodies were found to all the test antigens (experiments 1-7). Both "nonprecipitating" and precipitating antibodies (experiments 7-14) formed a specific precipitate with horse globulin and human sulfanylanthranylazoglobulin, i.e., in this series of experiments they behaved like trivalent compounds. An exception was the "nonprecipitating" antibodies to horse sulfanylanthranylazoglobulin (experiment 4), which reacted only with this particular antigen. Evidently this antigen most completely exhausted all the antibodies of the antiserum.

It should be pointed out also that, after being precipitated and separated, the "nonprecipitating" antibodies became, as it were, more avid and acquired the power of precipitating the antigens used for exhausting them.

It may be concluded from the experimental findings that the antibodies reacted with our test antigens like "multivalent" compounds. The particular "valency" of a chemically modified protein was evidently due to the number of specific groups introduced into the antigen. Thus "bivalent" antibodies appeared against monoazoproteins (see Table 1) and "trivalent" antibodies against diazoproteins (see Table 2).

The presence in an immune serum of a fraction of avid antibodies, strictly specific to the immunizing antigen, alongside "nonprecipitating" antibodies to the exhausting antigen only, indicated the presence of antibodies with varying tendencies to deviate from the mean degree of specificity. Antibodies which reacted more weakly with the corresponding antigen probably reflected its structural peculiarities less perfectly. This finding spoke against the hypothetical plurality of antibodies in a serum against a "polyvalent" antigen. It indicated the formation of a single antibody which reflected more or less clearly the whole complex structure of the antigen used for immunization. The reaction between the antibodies and heterologous antigens must evidently be explained by their structural similarity to the immunizing antigens and not by the presence of particular specific groups in the antibody. This similarity of the immunological specificity of different chemically modified proteins is due to the identical nature of the changes in the protein molecule resulting from denaturation, caused by the treatment itself and by the presence of the corresponding chemical radicals in the protein.

On the basis of the foregoing it can therefore be postulated that during immunization antibodies are formed which show specificity only to the antigen used. This specificity of the antibodies reflects more or less clearly the structure of the antigen. The "valency" displayed by the antibodies is due to the similarity of structure between the heterologous and immunizing antigens and also to the less complete specific correspondence between the individual structural peculiarities of the antigen and its antibodies.

SUMMARY

The "valency" of antibodies was studied by the method of exhaustion of antisera against mono- and diazoproteins, and subsequent cross reactions both with the antibodies left over in the supernatant fluid of the serum and with the "precipitating" and "nonprecipitating" antibodies isolated from the precipitate.

It was proved that the antibodies interact with the antigens as multivalent compounds.

The "valency" determined with regard to the azoproteins is dependent upon the number of groups introduced.

Thus, "bivalent" antibodies correspond to monoazoproteins and "trivalent" ones to diazoproteins.

The "valency" of antibodies is, evidently, determined by the structural similarity of the heterologous and the immunizing antigens as well as by the less complete specific conformity between the individual structural peculiarities of the antigen and its antibody.

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THE ROLE OF THE LYMPHOID ORGANS IN ANTIBODY PRODUCTION

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After extirpation of the thymus or the artificial production of hyperactivity of the thymus, many workers have observed the development of various changes in animals. It has been possible to show in this way a connection between the function of the thymus and the assimilation of vitamins [8], the influence of the thymus on the nervous system [9] and the musculature [2] and also a connection between this gland and other glands of internal secretion [1, 6, 10, 11, 12]. No unanimity yet exists, however, concerning the role of this gland in the body as a whole.

The problem of the role of the thymus as a lymphoid organ is of interest in connection with research in recent years into the importance of lymphoid tissue in immunogenesis. There is an extensive literature on the role of the spleen in the process of antibody formation, but so far as the thymus is concerned very little has been written on this subject. There are some observations on the lowering of the level of immunity in animals subjected to partial or total thymectomy, as revealed by lower morbidity and higher mortality in these animals by comparison with controls [1]. On the basis of experiments in which dry powdered thymus was fed to rabbits and mice, we know that hyperthymization inhibits the growth of mice and lowers the immune properties of rabbits. Finally there are results [5] showing a fall in the titer of antityphoid agglutinins in rabbits after receiving thymus extract.

The aim of the present research was to ascertain the effect of removal of the thymus and spleen on antibody production in rabbits and to discover the relationship between these two organs in immunogenesis.

EXPERIMENTAL METHODS

Investigations were carried out on rabbits weighing from 2 to 3 kg. Thymectomy and splenectomy were performed under ether anesthesia. The thymus was removed first, and the spleen 48 hours later.

The antigen used for immunization was a vaccine prepared from typhoid bacilli (strain 495), killed by heating to 56-58° for 1 hour.

The rabbits were vaccinated intravenously by means of triple immunization with doses of 1.2 and 2×10^9 bacterial cells at 7-day intervals between injections. Revaccination was by a single injection of 2×10^9 bacterial cells of the same vaccine.

The immunological response was judged by the rise in the agglutinin titer in the sera of the animals. Blood samples were taken before each injection of antigen and then subsequently once a week. The observations were prolonged until a fall in the titers, after which the animals were revaccinated in order to study the anamnestic reaction.

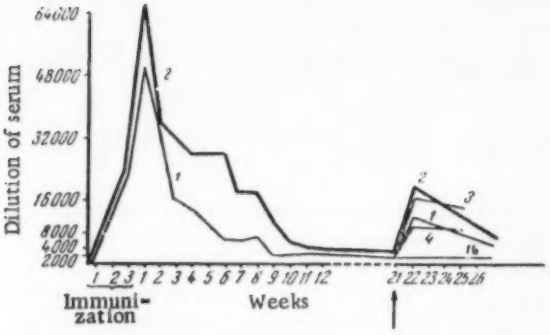

Fig. 1. The effect of thymectomy on the agglutinin titer in rabbits after triple immunization and revaccination. 1) In controls, 2) after removal of the thymus, 3) after removal of the thymus at the time of revaccination, 4) after removal of the thymus 48 hours after revaccination, 1b) not revaccinated. The arrow (↑) indicates the time of revaccination.

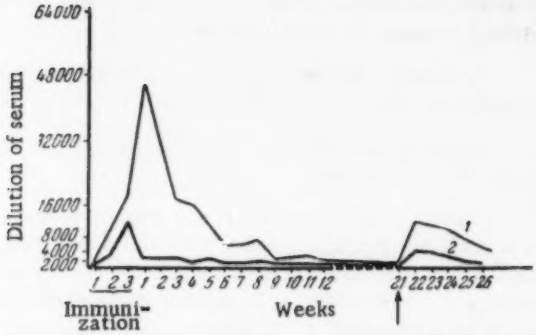

Fig. 2. The effect of splenectomy on the agglutinin titer in rabbits after triple immunization and revaccination. 1) In controls, 2) after splenectomy. The arrow indicates the time of revaccination.

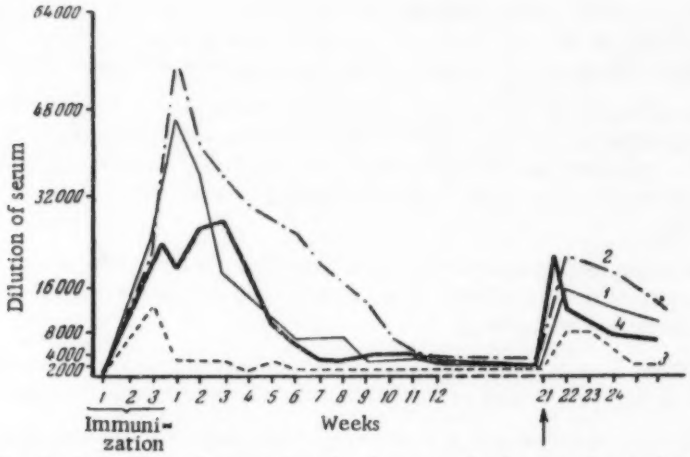

Fig. 3. The effect of removal of the thymus and spleen on the agglutinin titer in rabbits after immunization and revaccination. 1) In controls, 2) after removal of the thymus, 3) after removal of the spleen, 4) after removal of spleen and thymus. The arrow (↑) indicates the time of revaccination.

The agglutination reaction was carried out by the usual method. The antigen used in the reaction was a suspension of the same culture, killed with formalin. The preliminary results of the reaction were read after incubation of a mixture of serum and antigen for 2 hours at 37° and the final results on the day following standing of the mixture for 18-20 hours at room temperature. The intensity of the positive reaction was indicated by the number of plus signs.

Certain modifications of the method are given in the description of the experiments.

EXPERIMENTAL RESULTS

In the first series of experiments 15 rabbits were used, in 5 of which the thymus was removed one week

before the beginning of immunization, and 10 rabbits acted as controls. All the rabbits were immunized by means of three injections of typhoid vaccine.

The blood of all the animals was investigated before immunization for the presence of normal antibodies. No antibodies were found in serum diluted 1 : 100.

The blood was subsequently investigated before each injection of antigen, and once a week after the conclusion of immunization.

As may be seen from Fig. 1, the mean titer in the control animals throughout the entire period of observation was slightly lower than in the thymectomized animals (2). Revaccination on the 21st day showed that the antibody titer was increased still further in response to the repeated injection of antigen both in the rabbits from which the thymus was removed before the primary immunization (2) and in those from which the thymus was removed at the time of revaccination (3). If, however, the thymus was removed 48 hours after revaccination (4), the antibody titer in these animals was equal to the titer in control rabbits not undergoing operation (1). The antibody titer in the rabbits which were not revaccinated stayed at the previous level (Fig. 1, 1b).

These experiments showed that the presence of the thymus in an animal depresses to some extent the reaction of the animal in response to the injection of an antigen. This was shown by the lower degree of accumulation of antibodies in the blood in control rabbits by comparison with thymectomized rabbits.

In the second series of experiments observations were made on the antibody titers of 5 rabbits after splenectomy. Five unoperated animals were used as controls. The conditions of immunization and revaccination were the same as in the previous series.

These experiments revealed a clear difference between the accumulation of antibodies in the unoperated and splenectomized animals (Fig. 2). The agglutinin titer of the control animals (1) was many times higher than that of the splenectomized animals (2). This relationship was maintained after revaccination.

This difference in the titers was not, however, observed when the animals were immunized subcutaneously. These findings are in agreement with clinical observations on patients with splenomegaly [7]. It has been observed, for instance, that when patients after splenectomy are immunized intravenously with typhoid vaccine, the antibody titer is much higher in these patients than in healthy persons. This difference was not observed after subcutaneous vaccination.

The next series of experiments was conducted on animals from which the thymus and spleen were removed simultaneously. The experiments were carried out on 10 unoperated rabbits, 5 without the thymus, 6 without the spleen, and 5 without both thymus and spleen.

In spite of the opinion of some authors [4] that animals from which the thymus and spleen are removed simultaneously are not viable, we were able to make observations on such animals for a period of 1 year.

The following observations, shown in Fig. 3, were made after immunization and revaccination respectively. The largest accumulation of antibodies in the blood was observed in the group of thymectomized animals (2) and the smallest in the splenectomized group (3) by comparison with the control group (1). The titers in the group of animals without both thymus and spleen occupied an intermediate position (4).

The experiments to study the reaction of rabbits in response to injection of typhoid antigen after removal of the thymus, spleen, or both organs thus showed that these organs, along with other organs and tissues, take part in immunogenesis, but that the parts they play are different. Whereas removal of the thymus not only does not lower the content of circulating antibodies in the blood stream but even increases their accumulation, splenectomy lowers the antibody titer in response to intravenous immunization to a considerable degree, a fact which indicates the antagonistic relationship between these two organs in immunogenesis.

The immunological response of normal and thymectomized animals differs only slightly, but the spleen has only to be removed for the suppressing action of the thymus on the accumulation of antibodies to be sharply expressed. Evidence for this is given by experiments in which the thymus was removed from splenectomized animals (see Fig. 3), when the titers in these animals rose by comparison with those in the animals from which only the spleen was removed.

It may be assumed from these findings that the difference in the immunological response depends on the functional state of the lymphoid organs at the moment of immunization, and in particular of the thymus and spleen.

Preliminary experiments in which thymus homogenate was administered in the course of immunization confirmed the hypothesis that the thymus depresses the response reaction of the body to the injection of antigen. These findings are in agreement with the reports in the literature [1, 3, 5] that hyperthymization leads to a fall in the immunological response of the animal.

These results suggest that in the thymectomized animal both the accumulation of antibodies in the blood in response to injection of antigen, and the liberation of antibodies from the organs and tissues, take place to a greater degree, as is shown by the more pronounced anamnestic reaction in the thymectomized animals.

SUMMARY

Simultaneous removal of the thymus gland and spleen in rabbits does not affect the viability of these animals. Together with the other organs the thymus gland and the spleentake part in the response of the animal to the administration of the typhoid antigen. This participation is manifested in various forms: thymectomy promotes greater accumulation of the antibodies in the blood; splenectomy on the contrary reduces the titre: Simultaneous removal of the thymus and spleen results in a greater accumulation of antibodies than in the case of splenectomized animals, although it is slightly less than in the normal ones.

Thus one may assume that the thymus has a depressing effect on the mechanisms responsible for the accumulation of the antibodies in the blood; this effect is especially clearly manifested in the absence of the spleen. This is also confirmed experimentally by the increased titer of circulating antibodies in splenectomized rabbits after thymectomy, as well as by experiments with administration of thymus homogenate. The depressive effect of the thymus gland is manifested only during the first 48 hours after the administration of the antigen.

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THE MECHANISM OF IMMUNITY TO TETANUS TOXIN AT EARLY PERIODS AFTER ACTIVE IMMUNIZATION

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Studies of the dynamics of development of immunity after primary immunization against tetanus by several authors [1, 3, 4, 10] have shown that the resistance of animals to tetanus toxin or to a culture of tetanus spores develops before the appearance of determinate amounts of antitoxin or of its minimum concentration in the blood. In previous work [5], we also showed that the resistance of white rats after immunization with relatively large doses of adsorbed tetanus toxoid (200-500 units) could be demonstrated from the 6th day after immunization, whereas antitoxin could be found in the blood in determinate amounts (0.002 antitoxin units and over) only from the 8th day.

The aim of the present research was to study the factors responsible for creating the resistance of animals to tetanus toxin at early periods after immunization when no trace of antitoxin was present in the blood.

EXPERIMENTAL METHODS AND RESULTS

Experiments were carried out on white rats weighing 160-180 g. For immunization we used concentrated, purified tetanus toxoid, adsorbed on Al $(OH)_3$, of series No. 57 of the Institute of Epidemiology and Microbiology of the AMN SSSR, containing 1000 units/ml. The immunizing dose was 200 units (0.2 ml) and was injected into the muscles of the left calf.

In the first series of experiments it was found that when 15 M.L.D. of tetanus toxin was injected on the 7th day after immunization, the majority of animals survived (6-7 of the 10 rats), although the antitoxin titer in the blood at this time was below the minimum determinate level (less than 0.002 antitoxin units). All the unimmunized control animals died on the second day after injection of this dose of toxin.

The results obtained suggest that, despite the absence of antitoxin from the blood of the immunized animals, neutralization of the toxin took place in the body of the animals. In order to test this hypothesis, we carried out experiments to study the dynamics of the resorption of toxin into the blood stream after being injected intramuscularly into immunized animals. The dose of toxin was 15 M.L.D., and this was injected into the muscles of the left or right calf. The toxin content of the serum at different times after injection was determined by biological titration in white mice. In the determination of the toxin titer at each period, a mixture of equal volumes of the sera of 4-5 rats was used. The results of the experiments are shown in Table 1.

The results show that the toxin content of the blood of the rats receiving injections of toxin on the 5th day after immunization was almost the same as that of the unimmunized animals. When the toxin was injected on the 6th day after immunization, it appeared in the blood at the same time and in the same titer as in the control

TABLE 1

Content of Toxin in the Blood Serum of Immunized Rats at Different Times after the Intramuscular Injection of 15 M.L.D. of Tetanus Toxin

Day, after immunization, that toxin was injected	Content of toxin (in M.L.D. for mice) in 1 ml of serum			
	interval between injection of toxin and taking of blood for investigation			
	minutes	minutes	hours	hours
5th	0	0.5—1	1	4
6th	0	0.5—1	0.25	<0.25 (traces)
7th	0	0	0	0
Control (unimmunized rats)	0	1	1	4—6

TABLE 2

Determination of the Antitoxic Properties of the Serum of Rats Immunized with 200 units Adsorbed Tetanus Toxoid, 6 Hours after Injection of 5 M.L.D. of Tetanus Toxin

Serum	Result
Of immunized rats receiving 5 M.L.D. tetanus toxin	1 ml of serum neutralized 2 M.L.D. for mice of tetanus toxin
Of immunized rats receiving physiological saline instead of toxin	The serum possessed no neutralizing properties against 2 M.L.D. for mice of toxin
Of immunized rats receiving no injections of any sort	The same
Of normal (unimmunized) rats	The same

animals, but subsequently (after 3 hours and especially after 6 hours) its titer fell considerably below that of the controls. On the 7th day after immunization, toxin, injected intramuscularly, was not generally found in the blood. Nevertheless, the serum of immunized animals, not receiving injections of toxin, possessed no toxin-neutralizing properties on the 6th and 7th day after immunization when investigated in vitro.

The experiments thus showed that, despite the absence of determinate amounts of antitoxin from the blood of animals at early periods after immunization (6th-7th day), neutralization of toxin took place in the animal body. The supposition arose that this neutralization might be due to antitoxin entering the blood after the injection of the toxin. That this was possible was shown by the fact that toxin, when injected into the animals on the 6th day after immunization, was absorbed without hindrance into the blood at the site of injection, although subsequently its content in the blood fell sharply.

In order to test this hypothesis we carried out experiments to determine the antitoxin in the blood of immunized rats after receiving injections of toxin. White rats were immunized with 200 units adsorbed tetanus toxoid. On the 7th day after immunization the rats were given an injection of 5 M.L.D. of tetanus toxin, and 6 hours later the toxin-neutralizing properties of the serum were investigated in vitro. The results of these experiments are shown in Table 2.

It is apparent from the results described that 6 hours after injection of the toxin the serum of the immunized animals possessed antitoxic properties; the serum of immunized animals receiving injections of physiological

TABLE 3

Determination of the Antitoxin in the Serum of Rats Immunized with 200 Unitsof Adsorbed Tetanus Toxoid, 6 Hours after Injection of Various Substances

Substance injected	Dose	Experimental animal no.	Antitoxin titer (in antitoxin units)
Tetanus toxoid	50 units	1	>0.02
		2	>0.02
		3	0.005
		4	≥0.005
		5	>0.002<0.005
Perfringens toxoid	40 units	6	0.005
		7	>0.005<0.01
		8	>0.02
Cortisone	0.05 mg	9	>0.01<0.02
		10	>0.005<0.01
	5 mg	11	>0.02
		12	>0.02
		13	>0.02<0.03
		14	>0.005<0.01
		15	>0.01<0.02
Physiological saline	0.5 ml	16	<0.002
		17	<0.002
		18	<0.002
		19	<0.002
		20	<0.002

saline instead of toxin, in the same volume, or no injections at all did not possess these properties, which was in agreement with earlier findings [5]. The appearance of antitoxin in the blood of the animals was thus connected with the action of the tetanus toxin injected into the animals.

In a final series of experiments we investigated the possibility of reproduction of the phenomenon of appearance of antitoxin in the blood by the action of various substances. For this purpose white rats were immunized with 200 units of adsorbed tetanus toxoid, and on the 7th day after immunization they received the following by injection: specific antigen – concentrated, purified, unadsorbed tetanus toxoid; nonspecific antigen – perfringens toxoid; cortisone which, according to the literature [11], plays an important role in the mechanism of the stress reaction. The antitoxin content of the serum was determined by the usual method of biological titration in mice. The results obtained are shown in Table 3.

The results of these experiments show that at early periods after immunization (the 7th day) white rats possessed the ability to react in response to the injection of various substances by the rapid appearance of antitoxin in the blood.

The problem of the site of formation of tetanus antitoxin (from whence it presumably enters the blood in response to the action of various factors) has not been specially investigated. In a series of experiments on white rats, however, we showed that, at early periods after immunization (6th-7th day), in the absence of antitoxin from the blood, the regional iliac lymphatic glands corresponding to the site of injection of the toxoid for immunization, possessed the power to neutralize any toxin which reached them – this was evidence of the production of antitoxin in these lymphatic glands. The results of these investigations, in agreement with data in the literature [2, 6, 7, 8, 9] on the site of formation of tetanus antitoxin in the body, suggest that the appearance of antitoxin in the blood in response to the action of various factors is connected with its entry into the blood stream from the regional lymphatic apparatus.

The findings thus show that one of the factors responsible for creating the resistance of white rats to tetanus toxin at early periods after primary immunization, in the absence of any determinate amount of antitoxin in the blood at the time of injection of the toxin, is the rapid delivery of antitoxin to the blood stream from its site of formation as a result of the action of various specific and nonspecific stimuli on the animal body, and especially of the tetanus toxin itself.

SUMMARY

Experiments on white rats have established that after primary immunization with a single, relatively large dose of adsorbed tetanus toxoid the resistance of the animals to tetanus toxin develops prior to the appearance of antitoxin in the blood. However, the administration at that period of various substances, such as tetanus toxin and Bac. perfringens toxoid, or cortisone, makes the animals react by rapid production of antitoxin in the blood in detectable amounts. This phenomenon is regarded as a nonspecific stimulation of the antitoxin-forming apparatus (regional lymph modes) periods after immunization and in the absence of antitoxin in the blood at the moment of toxin administration.

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EXPERIMENTAL BIOLOGY

THE PHYSIOLOGICAL REGENERATION OF THE MAMMALIAN LIVER

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According to the results of many investigations, mitoses are either not found in the liver of adult mammals [8] or they are found in very small numbers: 1 mitosis per 10,000-20,000 cells [7, 3]. In this connection, the problem of the method of renewal of the cellular composition of the liver remains unsolved. Some research workers consider [8, 13, 14] that the liver is a "stable" organ in which no replacement of cells takes place, while others [3, 8, 12] incline to the view that liver cells die in the process of the vital activity and are then replaced with new cells, formed as the result of amitotic division. Insufficiently clear data has, however, been submitted in support of this point of view.

Our attention was attracted to work in which the diurnal rhythm of mitotic activity was studied in various organs characterized by the presence of obvious proliferation. It was shown, in particular, [1, 2] that in rats and mice the mitotic activity in the cornea, the epidermis, and the mucous membrane of the small intestine was minimal at midday and increased sharply during the night and morning hours. We found a similar pattern in work which we carried out jointly with Z. A. Ryabinina [4], on the investigation of changes in the mitotic activity of the regenerating liver of the mouse. Studies of the effect of hypnotics on the mitotic activity of the liver cells of rats showed [6] that a large number of mitoses could be found during the hours of night in many of the control animals which were untreated in any way. There are reports of the diurnal periodicity of the mitoses in the liver during growth [15].

When examining the liver of animals acting as controls to mice used in the work cited above [4], our attention was also directed to the fact that mitoses could easily be found in some of them during the hours of morning and night.

These findings led us to postulate that the replacement of the liver cells in mammals is brought about by mitotic division, although this cell division takes place mainly during the night and morning, and by virtue of the fact that the majority of research workers have fixed the liver of their experimental animals during the daytime, they have been unable to find any mitoses.

In order to test this hypothesis, we carried out a special series of experiments in which animals were sacrificed at various times of the day or night, and then the mitotic activity of the liver cells was investigated.

EXPERIMENTAL METHODS

Eighty sexually mature male white mice, weighing on the average 22 g, and taken recently from the nursery, were divided into 8 groups, each of 10 mice. The animals were kept on ordinary rations and subjected to no form of interference. The mice were killed by decapitation at intervals of 3 hours throughout the 24 hours. The liver

TABLE

Mitotic Activity of the Liver Cells in Adult Mice at Different Times of Day and Night (November, 1958)

	Time of fixation of liver (hours)							
	12	15	18	21	24	3	6	9
Number of mitoses per 6000 cells in individual animals	0	0	0	3	0	1	2	4
	0	0	0	0	0	10	1	4
	0	2	0	1	0	0	9	0
	0	0	0	0	0	3	5	5
	1	1	2	0	0	1	0	1
	4	1	0	1	1	6	10	1
	1	1	1	0	0	4	18	1
	0	2	0	0	0	2	5	2
	1	0	1	2	1	0	15	0
	0	0	1	1	1	—	9	0
Average	0.7	0.7	0.5	0.8	0.3	2.7	7.4	1.8

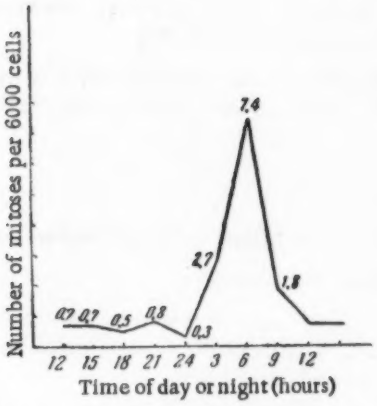

Fig. Changes in the mitotic activity of liver cells at different times of day and night.

was fixed in Zenker's fluid with acetic acid. A piece of the left lateral lobe of the liver, measuring 16-20 mm^2, was excised from the liver which weighed on the average 1300 mg. The pieces of liver were embedded in paraffin and sections were cut to a thickness of 6 μ and stained with hemalum and eosin. Mitoses were counted in 300 fields of vision (ocular 7, objective 90, the diaphragm in the ocular fitted with an aperture measuring 7 mm square). Under these conditions a field of vision contained about 20 cells, so that about 6000 cells were examined altogether. Twenty sections were always examined, each 8th-10th being selected, in order to prevent the same mitosis being counted twice. Only mitoses in liver cells were counted.

EXPERIMENTAL RESULTS

The results obtained are shown in the table and also illustrated graphically (see figure).

It can be seen from the figures in the table that the mitotic activity of the liver cells of the mice varied at different times of day and night. It was low in the period from 12:00 to 24:00 hours. In many animals at this time mitoses were completely absent and in the rest, as a rule 1-2 mitoses were seen. On the average there was 0.3-0.8 mitosis per animal (or more accurately per 6000 cells), which agrees fully with the findings in the literature. A different picture can be observed in livers fixed at later times: 3:00, 6:00 and 9:00 hours. At these times a large number of mitoses could be seen. Its average values were 2.7, 7.4, and 1.8 respectively. Mitoses were especially numerous in the group of mice at 6 a.m., when the mitotic activity was maximal. Of 74 nuclei of liver cells dividing at this time, 20 were at the prophase, 35 at the metaphase, 3 at the anaphase, and 16 the telophase. The phase ratio of the mitoses was similar to that observed during reparative regeneration of the liver, i.e., the greater number of nuclei were in the early stages of division [7]. Meanwhile, in some animals at this same period mitoses were rare or absent altogether.

It was shown by statistical treatment of the results obtained that the difference between the number of mitoses at 3:00 hours and at the preceding times of the experiment was significant ($P = 0.0000$). The difference

between the number of mitoses at 24:00 and 6:00 hours was also significant. The difference between the number of mitoses at 3:00 and 6:00 hours was close to significant (P = 0.03).

The findings suggest that our hypothesis was confirmed. The mitotic activity in the liver of mice was reasonably well marked in the period from 3:00 to 9:00 hours. Accurate limits of the period of increased mitotic activity were not defined, for the intervals of time used in the investigation were large, namely 3 hours. Mitotic activity, although on a far lower scale, was also present at the other times of day and night. Rough calculations show that, if the duration of mitosis in the liver cells is taken to be 1 hour, the cell complement of the liver may be completely renewed in 100 days. The rate of physiological regeneration in the liver is slower than in some other organs, but the replacement of its cells is an undoubted fact.

It must be pointed out that we could find no special "cambial" areas in the liver, in which particularly intensive division of liver cells might take place. Mitoses were found everywhere throughout the lobules, and no tendency was shown for them to accumulate at the periphery. Replacement of the Kupffer cells, the cells of the stroma, and the cells of the bile ducts took place as well as of the liver cells, as was shown by the frequent discovery of mitotic figures of division of their nuclei. We therefore consider that there are no grounds for regarding the liver as a "stable" organ, in view of its physiological regeneration.

It is interesting that the curve which we obtained showing the changes in mitotic activity of the liver cells was extremely similar to the curves of mitotic activity of the other tissues investigated by M. T. Gololobova [1, 2].

Naturally, the discovery of mitotic activity in the liver cells does not disprove the possible occurrence of amitotic division in the liver, but the detection of amitosis is difficult on account of the lack of precise criteria of their presence. The diurnal differences in mitotic activity may be to some extent associated with findings of diurnal changes in the biochemical and histochemical indices of the normal liver [5, 9, 10].

The discovery of the method by which the physiological regeneration of the normal mammalian liver is effected makes it imperative that the diurnal changes in the mitotic activity of other organs be studied, in which no mitoses are found during the daytime.

SUMMARY

Sexually mature white mice, weighing 22 gms, were sacrificed every 3 hours within the 24-hour period.

Mitoses in the liver were counted in 300 fields of vision (in about 6000 cells).

The mitotic activity is very low during the period from 12:00-24:00 hours. It is increased at 3:00 hours, reaches its maximum at 6 (7.4 mitoses) and is reduced at 9:00 hours. These data demonstrate that replacement of cells in the liver occurs by mitotic division during the night and morning hours.

The rise of mitotic activity during the period from 3:00 to 9:00 hours is statistically significant.

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THE ACTION OF FOLLICULIN AND PROGESTERONE ON THE MITOTIC ACTIVITY OF THE UTERINE EPITHELIUM IN MICE

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In our previous researches [2, 4] we showed that the mitotic activity of the epithelium of the uterine mucous membrane in mice undergoes regular changes during the sexual cycle and can be represented graphically in the form of a curve with the largest number of cell divisions in proestrus and in the transition to diestrus.

The first increase in mitotic activity in proestrus is evidently due to an increase in the concentration of estrogens in the blood. There are reports in the literature [1, 7, 8] of the stimulating action of estrogens on cell division in the epithelium of the uterine mucosa. It has not yet been explained, however, what causes the rapid fall in the mitotic activity and its maintenance at a low level during estrus, when the blood folliculin level continues to remain high.

Even less clearly understood are the principles involved in the mechanism of the second increase in mitotic activity in metestrus, when the blood estrogen concentration falls. The problem of the role of progesterone in this increase has necessitated special investigation, for the corpora lutea in rats and mice, according to the existing opinion [6], becomes functionally active only after mating or after the action of certain other factors. Attempts to investigate this problem during natural activation of the corpora lutea in mice [3] did not reach any definite conclusions. It was found that at early periods of pregnancy the mitotic activity of the uterine epithelium is no higher than at the same periods after ovulation in nonpregnant mice. Meanwhile, there are reports in the literature [9, 10] that progesterone may exert a stimulating action on division of the cells of this tissue.

In the present work we investigated the action of the sex hormones – folliculin and progesterone – on the mitotic activity of the epithelium of the uterine mucosa in mice.

From analysis of our previous results [2, 3, 4] and also from data in the literature [5] it is considered that the influence of the sex hormones on cell division even within the confines of the reproductive system is much more complex than is apparent from the existing information, and that the final effect of the action of the hormone depends on the state of the tissue under investigation.

EXPERIMENTAL METHODS

For the experiments we used 130 sexually mature female mice, weighing 22-23 g. Ovariectomy was performed on all the animals, which were then divided into 8 groups with 8-10 experimental and 7 control animals in each group. Starting on the 20th day after removal of the ovaries, the experimental mice of groups I-V received daily 25 units (2.5 γ) of folliculin in 0.1 ml of apricot oil. The animals of these groups were given different doses of this preparation (2, 4, 6, 8, and 10 injections). The control mice received the same number of

TABLE 1

Mitotic Coefficient in the Uterine Epithelium of Mice Receiving Injections of Progesterone

Hormone used	No. of animals	No. of injections	MC
Progesterone	9	5	0.36
Progesterone after 6 injections of folliculin	10	5	0.08
Folliculin	8	6	0.05

TABLE 2

Number of Mitoses by Phases in a Conventional Unit of Area of Section

Nature of experiment	Area of epithelium in conventional units	No. of mitoses per 1000 conventional units	MC	Phases of mitosis						PC
				early prophase	prophase	metaphase	anaphase	telophase	late telophase	
2 injections of folliculin	3 000	267.2	0.30	86.3	28.3	67.3	23.7	28.3	33.3	2.1
4 injections of folliculin	3 670	182.3	0.16	81.7	21.2	34.9	11.9	16 3	16.3	3.1
6 injections of folliculin	2 800	54.4	0.05	24.6	7.8	12.1	2.1	5.0	2.8	4.4
8 injections of folliculin	2 840	70.4	0.06	22.5	8.8	25.0	3.5	8.1	2.5	4.0
10 injections of folliculin	3 700	94.8	0.08	40.5	14.3	19.5	5.7	8.9	5.9	3.6
Cessation of folliculin injections	690	371.8	0.38	131.9	34.8	131.9	28.9	36.2	8.1	4.0
Injection of progesterone after 6 injections of folliculin	2 600	93.8	0.08	29.6	15.4	30.0	6.1	8.1	4.6	3.9
Injection of progesterone	1 600	336.2	0.36	88.7	33.8	123.7	21.9	45.6	22.5	2.7

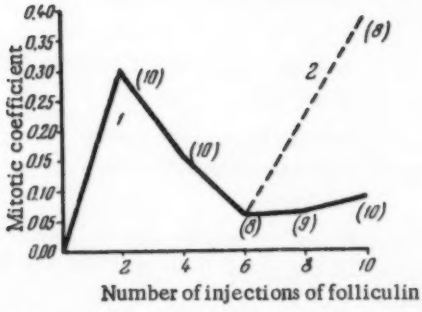

Fig. Changes in the mitotic coefficient in the uterine epithelium of mice. 1) During injection of folliculin; 2) after cessation of folliculin injections. The figures in brackets represent the numbers of experimental animals.

injections respectively of oil. The animals were sacrificed 24 hours after the last injection. The animals of group VI received 6 injections of folliculin and they were then sacrificed 5 days after the last injection. After receiving 6 injections of folliculin, the animals of group VII were given injections of 0.2 mg progesterone in 0.1 ml oil daily for 5 days. The animals of group VIII also received progesterone injections for 5 days, but after 6 injections of oil. The animals of groups VI-VIII were sacrificed simultaneously.

The effectiveness of action of the preparations was checked throughout the whole experiment by taking daily vaginal smears. The animals were killed by decapitation, all at the same time of day (11.00-12.00 hours).

The uterus was fixed in Zenker's fluid. Serial transverse sections were cut through the upper half of the right cornu of the uterus, to a thickness of 7 μ,

and stained with Carazzi's hematoxylin; the number of mitoses in the epithelium of the mucous membrane was counted in 10 sections (at intervals of 3 sections), as described in previous papers [2, 4].

The mitotic coefficient (MC) was determined as the number of mitoses per unit area of epithelium. The degree of significance of the differences observed was calculated by the Fisher-Student method.

EXPERIMENTAL RESULTS

Only isolated mitoses were found in the uterine epithelium of the ovariectomized mice whatever the time of sacrifice. After only 2 injections of folliculin (see figure) the number of cell divisions reached considerable proportions (MC = 0.30). With further injections, however, the mitotic coefficient fell; after 4 injections MC = = 0.16 and after 6 injections MC = 0.05; the fall in the number of mitoses in the interval between the second and 6th injections was significant (P = 0.01). This low value of the mitotic coefficient was maintained, with slight fluctuations, until the end of the course of injections of folliculin. With cessation of the administration of folliculin at the 6th injection (group VI) there was a sharp rise in the number of cell divisions (MC = 0.38, $P < 0.0001$).

The administration of progesterone to ovariectomized mice (Table 1) also had a considerable stimulating action on mitosis in the uterine epithelium (MC = 0.36). During the action of progesterone after preliminary administration of folliculin to animals, the mitotic coefficient remained almost the same (MC = 0.08) as after the 6th injection of folliculin (MC = 0.05).

Besides determining the mitotic coefficient, we also calculated the phase coefficient (PC), i.e., the ratio between the sum of the early phases of division (early prophase, prophase, and metaphase) and the sum of the late phases (anaphase, telophase, and late telophase).

As shown by Table 2, the value of PC more than doubled as the mitotic coefficient fell between the second and 6th injections of folliculin. Analysis of the causes of the changes in PC suggested that the fall in the value of the mitotic coefficient after two injections of folliculin was to a large extent connected with acceleration of the course of the late phases of mitosis, since the number of cells engaging in division was only very slightly less after 4 injections than after 2 injections of folliculin.

After 6-10 injections of folliculin, when the mitotic coefficient was very low, the number of early phases of mitosis became smaller, although PC rose to 4.4. Obviously the course of later phases of mitosis was accelerated in addition to the onset of depression of impending cell division. Cessation of administration of folliculin after 6 injections, when PC was 4.4, led to a sharp and proportional increase in all phases of mitosis (PC = 4). In this case, therefore, there was a universal stimulation of mitotic activity.

During administration of progesterone after 6 injections of folliculin approximately the same trend of changes in the phases was observed as after 8 and 10 injections of folliculin. The phase coefficient during the action of progesterone alone was close to that which was found after 2 injections of folliculin, apart from the number of metaphases, which was doubled.

The changes observed in the mitotic activity were accompanied by definite morphological changes affecting the uterine mucous membrane throughout the period of administration of the estrogen.

On the 20th day after ovariectomy the uterus was very small and its lumen narrow. The epithelial cells were cubical and sometimes flattened, their nuclei round or polymorphic and often shrivelled. The stroma was very compact and the glands feebly developed.

After 2 injections of folliculin the uterus increased sharply in size; its cavity was large and its contours had become indented. The epithelial cells were high-prismatic and their nuclei elongated, staining intensively and uniformly. The stroma was friable and a small number of glands with a well marked lumen was observed.

After 4, and occasionally after 6 injections of folliculin, the uterus in the majority of cases was elongated and its cavity had attained a very large size; secretion was often present in the cavity. The epithelial cells were cylindrical and cubical, with round or polymorphic nuclei. The glands were well developed.

Starting after 6, and especially after 8 and 10 injections of folliculin, the borders of the cavity became broken up and the epithelial layer composed of several rows of cells, arranged in folds, or sometimes it became syncitial in character. Pyknosis and rhexis of the nuclei were frequent. Vacuolation of the epithelium was seen

with desquamation of its cells and sometimes of whole areas into the lumen; occasionally a marked leucocytic infiltration could be observed. The glands developed to an extraordinary degree, and in isolated cases they showed cystic hyperplasia.

On the 5th day after cessation of the folliculin injections the uterus had almost regained its original size. The epithelial layer became less complex, the stroma was compact and the glands reduced in size.

In the normal sex cycle the process of destruction of the epithelium begins at the end of estrus and reaches its full development in metestrus; at the same time regeneration of the epithelium is proceeding, its reconstruction being completed in diestrus. At this time the blood estrogen concentration is known to be decreased. Our experiment after cessation of folliculin administration was to some extent an attempt to reproduce this part of the sex cycle. The high mitotic activity observed under these conditions was evidently connected, as in the normal animal, with regeneration of the epithelium and restoration of its original state. It is natural that, during the normal cycle, when the folliculin concentration falls gradually, the changes in mitotic activity are themselves more gradual in character.

This comparison thus confirms our previous hypothesis [2, 3, 4] that the mitotic activity is closely connected with the functional state of the organ.

The uterus of those mice which received injections of progesterone after folliculin, showed characteristic changes, known as progestin changes [6]. The uterine cavity was relatively large in size. The surface of the epithelial layer became nodular in appearance. The epithelial cells were mainly high-prismatic and their nuclei elongated and uniformly stained. The stroma was compact close to the lumen and the glands well developed.

When progesterone was given to the ovariectomized animals without previous treatment with folliculin, the size of the uterine cavity remained small. The epithelial cells were cylindrical and their nuclei oval. The stroma was very compact and the glands negligible in number and reduced in size. The experiment in which progesterone was injected without previous folliculin administration gave results suggesting that the mitotic activity is a more sensitive index of the tissue reaction than its proliferative changes. This hypothesis is in agreement with the experimental findings of Haskins and Leong [7], who observed the highest mitotic activity in the uterine epithelium of rabbits 48 hours after injection of progesterone, whereas the full completion of proliferation required 72-96 hours.

The results obtained suggest that folliculin has a stimulating effect on mitotic activity only after the first injections of the drug. Continuing saturation of the tissue with the hormone not only causes no further increase in the number of dividing cells, but, on the contrary, it leads to a fall in their number. Cessation of the folliculin injections at a time when its possibilities of stimulating this particular tissue are evidently exhausted, leads to an increase in mitotic activity.

Progesterone also had a stimulating action on cell division in the uterine epithelium. Its stimulating action in the conditions of our experiments, however, was observed only when no preliminary injections of folliculin had been given to the animals.

There are, therefore, at least two hormonal stimulators of the mitotic activity of the uterine epithelium, the effectiveness of which differs in accordance with the initial state of the tissue.

SUMMARY

Only individual mitoses are observed in the epithelium of the uterine mucous membrane of mice on the 20th day after ovariectomy. Following 2 folliculin injections the number of cell divisions is considerably increased; after 6 injections the number of mitoses becomes diminished and this reduction holds with further administration of the hormone (up to 10 injections).

Injections of progesterone to ovariectomized mice also have a considerable stimulating effect on cell divison in the uterine epithelium. If progesterone is administered after preliminary folliculin injections no such effect is observed.

Consequently, there are at least two hormone stimulants of mitotic activity of the uterine epithelium, possessing different degrees of efficacy depending upon the initial condition of the tissue.

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THE STUDY OF THE IMMUNOLOGY OF EMBRYOGENESIS

COMMUNICATION II. PHAGOCYTOSIS OF THE RED CELLS OF CHICK EMBRYOS BY LEUKOCYTES OF ADULT FOWLS

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At the end of the last centruy I. I. Mechnikov [6, 7] and A. O. Kovalevskii [5] demonstrated the important role of the phagocytes in processes of metamorphosis of the Echinodermata, Amphibia, and Insecta. These findings were later confirmed by the researches of other authors [1, 2, 10, 11, 12]. As a result of this research, Mechnikov [8] expressed his views on the morphogenic importance of the reaction of phagocytosis.

On the basis of these findings and of certain theoretical considerations, N. N. Zhukov-Verezhnikov [4] put forward his theory of primary immunological reactivity. One of the premises of this theory states that in the course of individual development certain definite immunological relationships arise between the protein systems of the tissues of different levels of development.

During the experimental investigation of this aspect of the theory, one of us, [3] was able to show that the tissue fluids of chick embryos contain substances which behave as antibodies in the complement fixation reaction with tissue extracts of chick embryos at earlier stages of development. These findings suggested that the immunological relationships between the tissues at different levels of development manifest themselves as humoral reactions of the antigen – antibody type.

We considered it of interest to ascertain by direct experiments whether these relationships were also manifested in the form of cellular reactions, and in particular of the reaction of phagocytosis. In order to solve this problem we carried out the experiments described below, in which we studied the phagocytosis of the red cells of chick embryos by the leukocytes of adult fowls.

EXPERIMENTAL METHODS

In order to obtain leukocytes from fowls one of the carotid arteries was opened (in some cases blood was taken from the comb). Clotting of the blood was prevented by the addition of a 2% solution of sodium citrate. The citrated blood thus obtained was centrifuged slowly (on a manual centrifuge) for an hour. As a result the red cells were deposited at the bottom of the tube and the leukocytes remained in the supernatant fluid (in the plasma), which was carefully aspirated with a Pasteur pipette*. In later experiments we used Sorokin's method [9], as follows. A ring of filter paper was placed on the surface of the yolk of a hen's egg after incubation for 52 hours**.

* To check completeness of deposition of the red cells, the supernatant fluid was examined under the microscope.
** The blood of 52-hour embryos is known not to contain its own leukocytes.

Fig. Macrophage with attached red cells, Magnification 900 X.

The yolk sac was carefully incised with ophthalmic scissors around the outer margin of the ring. As a result, when the ring was removed with forceps, the embryo was inside the ring. One drop of fowl plasma containing leukocytes was then placed on the ventral aspect of the embryo. The ring was then transferred to a chamber constructed by V. I. Sorokin, enabling in vivo observations to be made on the processes of development of chick embryos for periods of more than 24 hours. Under a microscope with a stage heated to 38-39°, observations were then made on the cells moving in one of the vessels of the embryonic disk. Studies were also made of the phagocytosis of the red cells of chick embryos incubated 3-4 days in vitro.

For this purpose a drop of blood from the embryo was mixed with a drop of fowl plasma containing leukocytes. This mixture was then placed in a watch glass on the heated stage and studied under the microscope.

EXPERIMENTAL RESULTS

The study of phagocytosis in vivo. Altogether 40 embryos were studied. To the ventral surface of 20 of these ambryos was applied a drop of fowl plasma containing leukocytes. The remaining 20 embryos acted as controls and were cultivated without the additionof plasma.

In all 20 experimental embryos, roughly 1-1½ hours after application of the plasma, active fowl's leukocytes penetrated through the vessel walls into the blood stream. They usually moved against the flow of blood, attaching themselves by their pseudopodia to the endothelial wall of the vessels.

When a phagocyte came into contact with any red cells moving towards it, the red cell was seized by the leukocyte and stayed near it. The red cell was so firmly held by the small protoplasmic processes of the leukocyte that it could not detach itself in spite of the strength of the current of blood and collisions with other red cells. In time the phagocyte was usually attached also to the wall of a vessel by a readily visible, large pseudopodium. Other red cells, colliding at this time with the phagocyte, were not attached to it. After the leukocyte, tightly held to the vessel wall by its pseudopodium, had once again resumed its round shape, the next red cell to come into contact with it was attached, and so on.

Having seized 3-4 red cells, the phagoctye detached itself from the vessel and passed into the general blood stream, where it evidently trapped new red cells on its way. In this manner (usually 2½-3 hours after the beginning of the experiment) were formed characteristic accumulations of cells which were often seen in the blood stream of the embryo. On closer examination (usually at the bifurcation of vessels, where these agglomerations of cells were usually held up) two types of cell could always be seen. In the center was the phagocyte, and around it were arranged the red cells attached to it (see figure). In other words the typical picture of the initial stages of phagocytosis was observed.

The process of phagocytosis did not always terminate in this way. The red cell seized by the protoplasmic processes was gradually drawn inside the phagocyte. From 6 to 8 hours after the beginning of the experiment macrophages could sometimes be seen with several ingested red cells. It must be pointed out, however, that it was not always easy to determine whether in fact the red cells were inside the macrophage, for the same appearance was presented by red cells situated beneath the phagocyte but not actually within its substance.

Besides macrophages, microphages were also concerned in the phagocytosis of red cells. In these cases, however, a smaller number of red cells was attached — usually 2-3.

The study of phagocytosis in vitro. Red cells from the blood of 10 chick embryos of 72-80 hours incubation were used in the experiment. It was found that the active leukocytes of the adult fowls exhibited positive taxis in relation to the red cells of the embryos immediately after their entry into the blood stream. Some 30-40

minutes after the beginning of the experiment the formation of characteristic agglomerations of cells could be observed, consisting of two types of cells: In the center was usually to be found an adult chick leukocyte, and around it were embryonic red cells, attached by small protoplasmic processes to the phagocyte. The number of these accumulations of cells gradually increased but no further development of the picture of early stages of phagocytosis took place in vitro.

From the results described it can thus be concluded that the primary immunological reactivity, i.e., the immunological relationship between the tissues when at different stages of individual development, also manifested itself in the form of the reaction of phagocytosis. It is interesting to observe that the process of phagocytosis of the red cells of chick embryos by the leukocytes of adult fowls followed a course which was absolutely analogous to the process of phagocytosis of foreign goose red cells, injected into the peritoneal cavity of a guinea pig, by the leukocytes of the recipient animal, as observed by I. I. Mechnikov.

The experimental findings suggest that the reaction of phagocytosis plays just as active a role in the process of embryonic development of birds as in the processes of metamorphosis of the Echinodermata [6], the Insecta [2, 5, 10, 11, 12], and the Amphibia [1, 7].

SUMMARY

The author studied the phagocytosis of red cells in chick embryos after 52 hours of incubation by the phagocytes of adult chickens. Experiments were conducted in vivo on the developing chick embryo to which fowl leukocytes were added, as well as in vitro, by mixing the red cells obtained from the embryo with the fowl leukocytes. The experiments demonstrated that fowl phagocytes behave with chick embryo red cells as with foreign cells, by effecting the initial stages of phagocytosis.

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THE INFLUENCE OF OVARIAN HORMONES ON GROWTH OF THE EPITHELIUM OF THE MAMMARY GLANDS

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According to the present-day views of the majority of oncologists and endocrinologists tumors of the mammary gland arise as the result of a disturbance of the balance of the hormones of the ovary and the anterior lobe of the hypophysis. Hormonal disturbances distort the proliferative and destructive processes in the epithelium of the mammary glands, as a result of which a tumor develops. In order to clarify the role of the individual hormones and of disturbance of their interrelationships in the processes of carcinogenesis, investigation of the hormal regulation of the growth of the mammary glands in normal conditions assumes great importance. This problem has been intensively studied in many countries [1, 2, 4, 6, 10, 11, 12, 16, 17, 18]. In the more recent work it has also been shown that all carcinogens producing carcinoma of the mammary glands possess to some degree the characteristic activity of a sex hormone, and some of them are very close in their chemical structure to the sex hormones [13]. It has been shown experimentally that the ovarian hormones – estrogens – stimulate proliferation of the epithelium of the mammary glands, and the hormone progesterone promotes formation of the secretory divisions of the glands [8, 9]. The lactigenic action of an extract of the anterior lobe of the hypophysis on the mammary gland was first discovered by Stricker and Grueter [19] in 1928. It was shown later, when the pituitary hormones had been separated and purified, that prolactin – a hormone from the anterior lobe of the hypophysis – – possesses a mammotropic action. Lyons [14] first obtained secretion of milk and observed active growth of the alveolar epithelium of the mammary gland after injecting prolactin directly into the gland.

Lyons and his coworkers later showed that the injection of ovarian hormones alone, without prolactin, into rats from which the ovaries, adrenals, and hypophysis had been removed, does not cause growth of the mammary glands [15]. When the growth hormone of the hypophysis had been purified, Lyons found that it acts directly on the mammary glands, causing some degree of stimulation of their growth, and that the activity of prolactin, estrogens, and progesterone is increased in its presence [16]. Later experiments led this worker to the conclusion that the normal development of the mammary glands requires the combined action of all these hormones, the prolactin of the hypophysis being the most important [17]. Lyons's findings were confirmed by Ferguson in experiments on mice [11].

In contrast to the conclusions of Lyons and his supporters, Ahren and Jacobsohn [7] showed that complete development of the mammary glands in hypophysectomized females may be indiced by ovarian hormones alone, i.e., in the absence of prolactin, if insulin is injected at the same time as the ovarian hormones. N. I. Lazarev also does not share Lyon's point of view and considers that gonadotropic hormone and estrogens have a dominant influence on growth of the mammary glands [4, 5]. Our work on the change in the mitotic activity of the epithelium of the mammary glands in pregnant and lactating mice [3] also contradicts Lyons's findings. We showed that the high mitotic activity of the epithelium of the mammary glands observed in the later stages of pregnancy

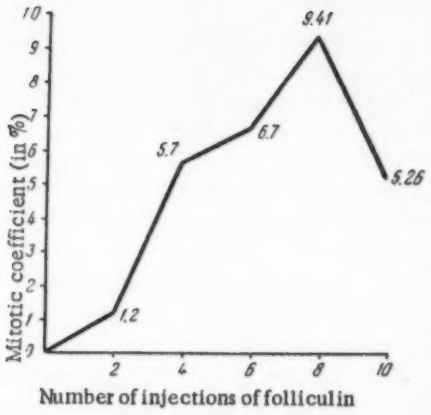

Figure. Change in the mitotic activity of the epithelium of the mammary glands after administration of folliculin to ovariectomized female mice.

falls sharply after birth and the onset of lactation. In this case prolactin, although it stimulates the secretion of milk, does not increase the mitotic activity of the epithelium. Much evidently depends on the conditions under which a given hormone acts and on the state of the cells which it affects. There is every reason to believe that the hormonal regulation of the growth of the mammary glands is more complex than might be gathered from the scheme suggested by Lyons. In recent years many papers have been published on the study of the action of the hormones of the adrenal cortex and the adrenocorticotropic hormone of the hyophysis (ACTH) on the mammary gland. The findings obtained are, however, contradictory. Flux [12] found that glucocorticosteroids inhibit the growth of the mammary glands in intact and ovariectomized animals. Selye [18], on the other hand, reported that ACTH and the glucocorticosteroids stimulate growth of the mammary glands and induce the secretion of milk.

We studied the effect of folliculin and progesterone on the processes of destruction and proliferation of the epithelium of the mammary glands in mice, using the method of determination of the mitotic activity. The results of these experiments are described in the present paper.

EXPERIMENTAL METHODS

Experiments were carried out on 122 sexually mature female mice. Bilateral ovariectomy was performed on all the animals in a period of 2 days. Twenty days after operation, when a persistent diestrus had become established in all the mice, a certain number of them were given daily subcutaneous injections of folliculin dissolved in apricot oil. The remaining control animals received daily injections of apricot oil. After 2, 4, 6, 8, and 10 injections of folliculin respectively, 9-10 experimental mice and 7-9 controls were sacrificed. The second and third right mammary glands were taken for histological examination. The mitotic activity of the epithelium of the terminal divisions of the glands was determined by the same method as in our previous research [2], and the mitotic coefficient was calculated (number of dividing cells per 1000). In one group (11 mice) administration of folliculin was discontinued after 6 daily injections. The 9 control mice to this group received 6 daily injections of oil and were sacrificed 5 days after the 6th injection. The action of the corpus luteum hormone — progesterone — on the intensity of cell division in the epithelium of the mammary glands was determined in two groups of animals. The first group of ovariectomized females received 6 daily injections of folliculin and then, on each of the next 5 days they were given a subcutaneous injection of 0.2 mg progesterone in 0.1 ml of oil. The animals were killed 24 hours after the last injection. The second group of ovariectomized females received 6 daily injections of oil and then, for the next 5 days, they were given subcutaneous injections of 0.2 mg progesterone in 0.1 ml oil.

EXPERIMENTAL RESULTS

After only the second injection of folliculin into the ovariectomized mice, diestrus was replaced by estrus. The vaginal smears of a small number of animals at this time still showed signs of the proestrus stage. After the third injection, all the mice save three were firmly in the stage of estrus. This provided convincing evidence of the activity of the preparation which we used. Determination of the mitotic activity in the control mice showed that ovariectomy completely arrested the mitotic division of the epithelial cells in the mammary glands, and that the act of injecting apricot oil subcutaneously did not itself cause an increase in the mitotic activity of these cells.

The mitotic activity of the epithelium of the mammary glands of the experimental mice increased considerably from one injection of folliculin to the next (see figure). After the second injection it rose on the average from zero to 1.2 mitoses per 1000 cells, and after the 4th injection it reached 5.7 mitoses per 1000 cells.

The differences between the mean values of the mitotic activity here were statistically significant ($P < 0.0001$). Although the mean mitotic activity after the 6th injection had increased to 6.7 mitoses per 1000 cells, comparison of this figure with the mean mitotic activity of animals sacrificed after 4 injections showed that this increase was not statistically significant. The further increase in the mitotic activity after 8 injections of folliculin was statistically significant ($P = 0.006$). In animals sacrificed after 10 injections of folliculin a considerable fall was observed in the mitotic activity of the epithelium of the mammary glands. Whereas the mitotic activity after the 8th injection averaged 9.41 mitoses per 1000 cells, after 10 injections it was only 5.26 mitoses per 1000 cells. The difference between these mean values was statistically significant ($P = 0.006$).

Histological investigation of the mammary glands in the control mice showed that bilateral ovariectomy causes marked atrophy of the glands. In our experiments the earliest time at which mice were sacrificed after ovariectomy was the 22nd day. At this and later times, no significant destructive changes could be seen in the gland for they had mainly been completed beforehand. Atrophy of the mammary glands after ovariectomy was shown as a considerable fall in the number of terminal divisions and a sharp decrease in the diameter of the major efferent ducts. The number of rows of nuclei in the epithelial lining of the terminal divisions and ducts was less than in the glands of normal nonpregnant mice. Among the nuclei of the cells of the terminal divisions there were many which were dark and pyknotic. The nuclei of the epithelial cells were smaller in size than in normal glands. Proliferative epithelial nodules, so characteristic of the progressively developing gland, were completely absent. Accumulations of desquamated epithelial cells could often be seen in the lumen of the ducts.

In the experimental animals sacrificed after 2 injections of folliculin the glands had the same structure as those of the control animals. However, the mitotic activity of the epithelium of the mammary glands in the control animals was equal to zero, and that of the experimental animals was increased by the action of folliculin to 1.2 mitoses per 1000 cells. The important conclusion thus followed that the general morphological examination of the structure of the mammary glands gives evidence of the action of the hormone much later than does the method of determination of the mitotic activity. After 4 injections of folliculin the morphological changes in the gland were now marked. Proliferative epithelial nodules were apparent. The epithelial lining of the alveoli and ducts was thicker and the lumen of the ducts wider. After 6 injections, and especially after 8 injections, the proliferative epithelial nodules continued to increase in number and size. This was evidently connected with failure of the cells, accumulating as the result of mitosis, to differentiate into typical organs.

The usual pattern of formation of bunches of alveoli, with a lumen and a single layer of epithelium, from the solid epithelial nodules, that is characteristic of the development of the glands in pregnancy was not observed. After 10 injections, although many proliferative nodules could be seen in the glands, the number of mitoses was appreciably reduced. After 8 and 10 injections secretory vacuoles appeared in the cells of the terminal divisions and the small ducts in a few animals. In the series of experiments in which ovariectomized females received only 6 injections of folliculin, after which the administration of folliculin ceased, the mitotic activity on the 6th day after the last injection fell to a negligible figure — 0.18 mitoses per 1000 cells. Evidently the mitotic activity did not fall to zero because the estrogens were gradually excreted from the animals. This was confirmed by examination of vaginal smears. In the first few days after discontinuation of the folliculin injections, estrus was observed in all the mice. After the 4th day diestrus supervened in some mice. Only on the 6th day was a state of diestrus found in all the mice.

Histological examination of the mammary glands revealed well marked signs of destruction. Proliferative epithelial nodules were absent. In many of the terminal divisions the epithelium had become separated from the basal membrane. Only the connective tissue framework remained of some alveoli. Many desquamated epithelial cells had accumulated in the fluid filling the ducts. The nuclei of the epithelial lining of the ducts showed considerable polymorphism. The nuclei of many epithelial cells were pyknotic. The number of leucocytes in the stroma of the gland and in its epithelial formations was perceptibly increased. In the control animals of this series of experiments the mammary glands were atrophied and no mitoses were present in the epithelial cells.

In the last series of experiments the animals received 6 daily injections of folliculin, followed by 5 injections of progesterone. The mean mitotic activity of the epithelium of the mammary glands in these animals was 0.97 mitoses per 1000 cells. This mitotic activity was much below the mitotic activity observed in normal mice in the stage of diestrus, but yet it was higher than in the previous series of experiments, the difference being statistically significant ($P = 0.006$). The results of the control experiments in which injections of progesterone followed

administration of oil were completely unexpected. In this case the mitotic activity was greater than in the experimental series, amounting on the average to 3.3 mitoses per 1000 cells. It was found that progesterone caused only a slight increase in the mitotic activity of the mammary glands, and its action was weaker when it was given immediately after folliculin.

In our previous research we showed that growth of the epithelium in nonpregnant mice with a normal sexual cycle and in the first days of pregnancy is similar in its character. This growth consists of the accumulation of epithelial cells by mititic division but with no differentiation of the typical epithelium. In the second half of the first week of pregnancy, differentiation of the typical organ is observed alongside profiferation. The epithelium enters into relationships with the connective tissue characteristic of the mammary gland, as a result of which the terminal divisions of the organ are formed. Alveoli with two rows of epithelial cells are formed from the epithelial anlage – the proliferative nodule [2, 3].

In our opinion, when speaking of growth of the epithelium of the mammary glands and of the regulation of this growth it is above all essential to know which of the two types of growth mentioned above is concerned. None of the authors cited in the survey of the literature have made such a distinction.

From the results of our experiments it may be concluded that the first type of growth is stimulated by estrogenic hormones. As our experiments showed, the presence of estrogens in the body is absolutely essential for mitotic division of the epithelial cells of the mammary gland. The repeated injection of folliculin into ovariectomized mice causes growth of the epithelium but does not stimulate the development of the gland as an organ, since no formation of alveoli takes place. The prolonged administration of folliculin ultimately leads to a lowering of the mitotic activity of the epithelium of the glands. We have no grounds at present for suggesting the courses of this lowering.

Lyons [15] and Ferguson [11] have shown that the injection of estrogens alone into animals after hypophysectomy does not cause growth of the mammary glands. Injection of prolactin alone under the same circumstances brings about growth of the epithelium with the formation of alveoli. From the illustrations in Ferguson's paper it can be clearly seen that the administration to ovariectomized and hypophysectomized females of estrogenic hormones in conjunction with growth hormone gives a well marked picture of the first type of epithelial growth. Evidently the second type of epithelial growth requires, in addition to hormones causing cell division, stimulation by differentiating hormones. In view of the findings of Cowie [10] and Lyons [15], prolactin and progesterone must be considered to be hormones of this type.

The apprehensions of Lyons and Ferguson that the adrenal cortex of ovariectomized females would begin to produce estrogens very soon after operation are greatly exaggerated. Our findings show that the adrenal cortex of mice 20–30 days after ovariectomy does not yet produce estrogens in amounts sufficient to cause keratinization of the epithelium of the vagina or mitoses in the epithelium of the mammary glands.

We were unable to confirm the observations of Cowie and Benson that progesterone causes obvious stimulation of the formation of alveoli in the mammary gland. In our experiments the administration of progesterone to ovariectomized mice led to the appearance of mitoses in the epithelium of the mammary glands, but this increase in the number of mitoses was insignificant by comparison with that due to the action of folliculin. We were not convinced that the progesterone preparation which we used was sufficiently active, for we did not carry out control tests of mucification of the uterine endometrium. In consequence of this, the results of the action of progesterone obtained in our experiments must be regarded as purely preliminary.

SUMMARY

The author studied the effect of ovarian hormones (folliculin and progesterone) on the mitotic activity of the terminal portions of the mammary glands in ovariectomized mice. He showed that the mitotic activity of the epithelium in the mammary glands increases from zero to 9.41 mitoses per 1000 cells after daily administration of folliculin in a dose of 25 I. U. to ovariectomized females. The maximal mitotic activity is noted after 8 folliculin injections. Following 10 folliculin injections the mitotic activity becomes reduced. In comparison with folliculin, progesterone provokes a lesser rise of mitotic activity.

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METHODS

AN APPARATUS FOR COMBINED DIELECTRO-BALLISTO-ELECTROCARDIOGRAPHY

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The functions of excitation and conduction of the cardiac muscle may be assessed very accurately and fully from the electrocardiogram; assessment of the contractile function of the heart muscle, characterized by the strength and volume of the systolic contractions, is much more difficult in clinical practice. The electrocardiographic findings are only of indirect significance in this direction; the ballistocardiogram gives more direct information. Some information on the pulsation of the heart and the great vessels is given by roentgenokymography, but the results of this form of examination are hardly comparable with those of electro- abd ballistocardiography. In the estimation of the functional state of the heart, the determination of the mechanical aspect of its activity thus remains the most difficult problem, and this calls for further improvements in technique.

One way of obtaining more direct information about the character of the cardiac contractions is to study the changes caused by these contractions in an external electric field applied to the thoracic region. Depending on the frequency of oscillation of the current of an electric field applied to the part of the body under study, changes in the volume of the organs situated in this part will be reflected by various physical indices. If a direct current is used, for instance, the pulse changes in the volume of the thoracic organs may be recorded as changes in the ohmic resistance together with the polarization capacity [7]. The use of an audiofrequency current [6] or especially a current of a frequency of the order of hundreds of kc [2] reduces the value of the polarization capacity factor and enables the results obtained to be regarded basically as changes in electrical conductivity [3, 4]. With a further increase in the frequency of the electric field, especailly when ultrahigh frequencies are reached (of the order of hundreds of Mc), capacitive conduction of current becomes possible, without having the electrode in contact with the body; in this case the pulsations of the thoracic organs are recorded as changes in the dielectric constant of the condenser formed by the electrode plates [5, 8]. Such a dielectrogram can give a very direct representation of the fluctuations in the volume of the heart and great vessels, the degree of their filling with blood and emptying, and also of other characteristics of the mechanical aspect of cardiac activity.

For the combined investigation of the functional state of the heart, we constructed an apparatus which enables parallel recordings of the dielectrocardiogram, ballistocardiogram, and electrocardiogram to be made. All working parts of the apparatus are fixed to a chair in which the patient sits in a natural position (Fig. 1). On the arms of this polycardiographic chair are cushions (1) soaked in physiological saline, on which the forearms are fixed, for the leads of the electrocardiogram. In the seat of the chair is mounted a system of piezoelectric pick-ups (2) for recording the ballistocardiogram, as suggested by A. V. Bestugin and his coworkers [1]. The electrocardiogram and ballistocardiogram can be recorded jointly in one recording channel by the method suggested by these authors.

The dielectrographic system (3) is assembled in accordance with the scheme outlined in Fig. 2. The system consists of a short-wave generator with a circuit connected inductively to it, the condenser of which acts as a pick-up. This condenser (4) consists of two metal plates fixed to the inside of the arms and the back of the chair,

Fig. 1. General view of the polycardiograph chair.

so that they surround the patient's chest on two sides (see Figs. 1 and 2). Individual differences between patients examined require that the generator be tuned every time by means of a variable condenser (5). This tuning is done while the patient breathes quietly, to a position at which the amplitude of the respiratory oscillations of the current of the generator observed on the microammeter (6) is maximal. Additional control with a variable resistor (7) enables the optimal conditions for the working of the lamp to be selected, by varying the anode voltage.

When the dielectrocardigram is to be recorded in conjunction with the ballisto- and electrocardiograms, the necessary conditions for the recording of the latter are first chosen, after which the generator circuit is tuned to the maximum amplitude of the respiratory oscillations of the current. When the optimum position has been found, the patient is asked to hold the breath in mid-inspiration and the recording is taken. A suitable recording system is a two-channel electrocardiograph, which is easily assembled from two type EKP apparatuses.

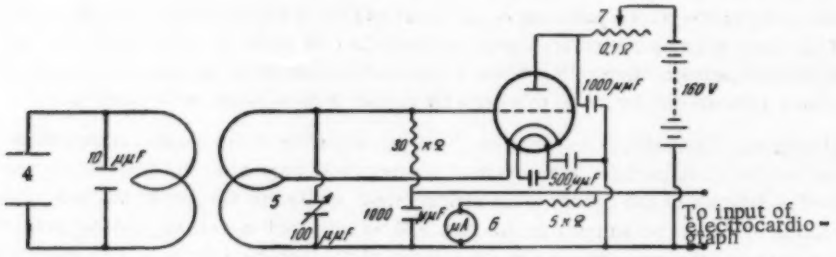

Fig. 2. Scheme of the dielectrographic part of the apparatus

The dielectrocardiogram consists of a combination of oscillations, reflecting the variations in the volume of the heart and of the great vessels during each cycle of cardiac contractions.

Fig. 3 shows the ratio between these oscillations and those of the ballistocardiogram to the waves of the electrocardiogram. A fall in the curve indicates a decrease in the volume as the result of the systolic expulsion of blood from the ventricles, which begins at the point 1 on the curve. At point 2 the decrease in the volume of the ventricles is compensated by an influx of blood into the atrium and by displacement of the heart. Point 3 corresponds to the end of ventricular systole and the beginning of the pause. During the pause the heart is filling with blood: quickly at first (to point 4) and then with small changes in volume.

The dielectrocardiogram shows considerable changes both in connection with the varying functional state of the normal heart and in different pathological conditions. Some examples of dielectrocardiograms of patients are shown in Fig. 3.

SUMMARY

The author describes the construction of an apparatus for combined dielectro-ballisto- and electrocardiography. The dielectrocardiogram reflects the pulsations of the heart and of the large blood vessels as changes of the dielectric constant in the condenser formed by metal plates between which the person under observation is sitting. Samples of recordings obtained in disturbances of the cardiac activity are presented.

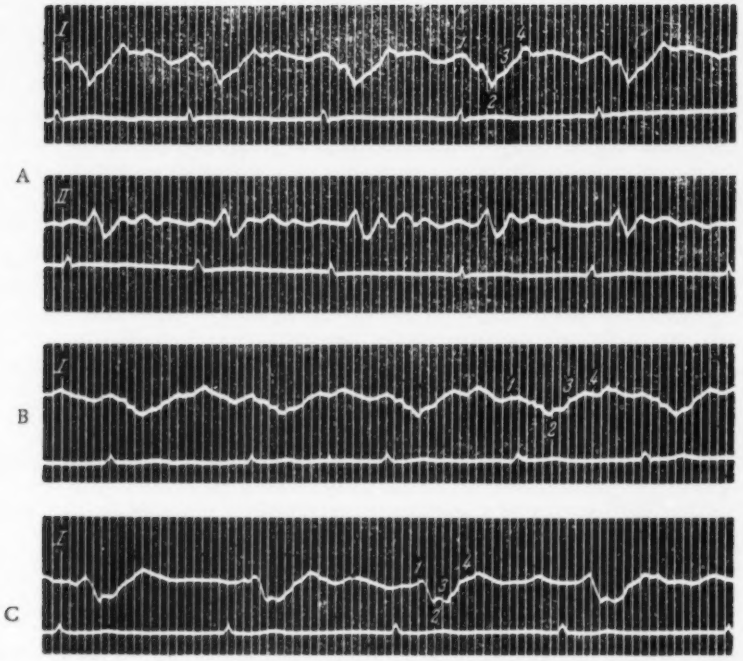

Fig. 3. Recordings of the dielectrocardiogram with the electrocardiogram (I) and the ballistocardiogram with the electrocardiogram (II). A) Patient A. L., aged 26 years, neurasthenia; B) patient R. P., aged 28 years, mitral incompetence; C) patient N. Sh., aged 39 years, stage II hypertension.

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A METHOD OF RECORDING THE RESPIRATION OF THE MAMMALIAN FETUS

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It is very difficult to record the respiratory movements of animals during the intrauterine period of development. This is primarily due to the small size of the fetus, and also to its imperfect thermoregulation, which necessitates the experiments being carried out in a bath of physiological saline warmed to the body temperature of the animal.

When studying the respiration of the fetus, the majority of workers have observed but not recorded the respiratory movements. Only a few workers such as Büdingen [3], and I. A. Arshavskii [1] have recorded the respiratory movements, for which purpose they inserted a hollow needle into the interpleural space, the free end of the needle being connected to a Marey's capsule by means of a rubber tube. This system was filled with water. Methods of recording the respiratory movements by means of a glass cannula or a rubber pipette, inserted into the esophagus through an incision in the neck, have also been published. These methods of recording inflict trauma on the fetus and do not provide good tracings of respiration during general motor reactions.

In view of these drawbacks, we adapted methods used in our laboratory for recording respiration in the postnatal period of development to make them suitable for recording respiratory movements in the fetus.

Each of the two variants of the method suggested enable respiratory movements of the rabbit fetus to be recorded from the moment that they first appear, i.e., from the 20th-22nd day of intrauterine development, when the weight of the fetus is 3-6 g. This method may be used also for recording respiration in the fetuses of other mammals.

1. Registration of the fetal respiration by means of the thermistor.

For this purpose a conical mask was turned from organic glass (Fig. 1), which concentrated the flow of inspired and expired air and protected the thermistor from the effect of extraneous air currents. The thermistor was fixed in a special opening at the narrow end of the mask (Fig. 1, b). The part of the thermistor inserted in the orifice of the mask, part of the wire, and the place where the wires leading from the thermistor to the Wheatstone bridge were soldered (Fig. 1, c) were all embedded in liquid organic glass.

The fetus was extracted from the abdominal cavity of the mother and then placed on a special organic glass stand (Fig. 2), on which was a hollow (a) which enabled the head of the fetus alone to be kept in the air, and a cut out portion (b) preventing compression of the umbilical cord through which the fetus maintained its connection with the mother.

The mask with the thermistor was applied over the buccal extremity of the fetus and fixed by means of a thin rubber band (Fig. 1, d) which passed from two diametrically opposite holes in the walls of the wide part of the

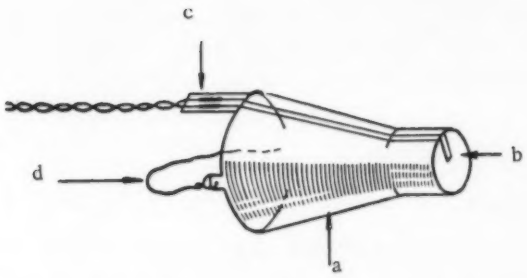

Fig. 1. Mask (a) for recording fetal respiration by means of a thermistor. b) Thermistor; c) point where leads are soldered to wires from thermistor; d) rubber band for fixing mask on head of fetus.

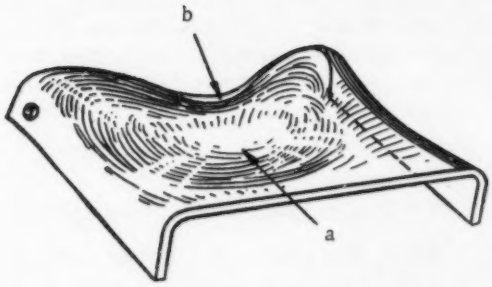

Fig. 2. Stand on which the fetus lies during the experiment. a) Hollow for fetus; b) piece cut out to prevent compression of the umbilical cord.

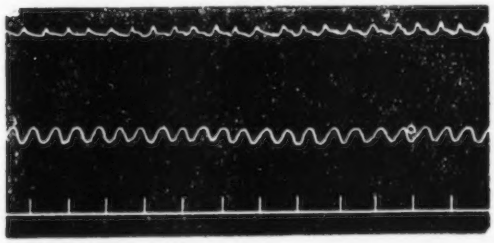

Fig. 3. Recording of respiration in the rabbit fetus. Significance of the curves (from above down): tracing of respiratory movements by the piezocrystal; the same, by the thermistor; time marker (in seconds).

mask, under the ears, and around the occipital part of the fetal head. The thermistor recorded the changes in the temperature of the inspired air by means of the ink-recorder of an electroencephalograph. A detailed technical account and the scheme of the method of recording the respiration by means of the thermistor are given in the article by A. A. Volokhov, V. I. Kobysh, and E. G. Novikova [2].

2. Recording the respiratory movements of the fetus by means of the piezoelectric pick-up.

The ordinary piezocrystal from a pick-up, the metal leads from it, and the point where the leads were soldered to it were coated with several layers of celluloid (cinefilm with the emulsion washed off), dissolved in acetone (thickness of covering (0.5 mm). During recording the piezocrystal was placed in the hollow of the stand for the fetus, beneath its thorax, and responded to all its movements. The currents resulting from deformation of the piezocrystal were amplified and recorded with the ink-recorder of an electroencephalograph. In order to prevent rapid overshoots, a condenser (with a capacity of 4μ in our experiments) was connected in the circuit of the piezocrystal.

In both cases, when the system was assembled, fine multicore leads covered with vinyl chloride insulation (diameter of wire 1 mm) were used. In both methods it is essential that physiological saline should not fall on the uninsulated parts of the thermistor, piezocrystal, or leads, and so they must be carefully insulated with organic glass or celluloid.

This method enables an objective analysis to be made of the respiratory movements of the fetus: their rate, depth and duration, and the pause between the separate phases of respiration. Fig. 3 shows the recording of the respiration of a rabbit fetus on the 29th day of intrauterine life, after cessation of the blood flow in the umbilical cord.

The piezocrystal also records the motor reactions of the fetus, but when this happens the tracing of the respiratory movements is distorted or interrupted. The motor reactions do not affect the registration of respiration by means of the thermistor. It remains only to mention that the mask with the thermistor must not be immersed in the physiological saline while the recording is being made.

SUMMARY

The author recommends a method of recording the respiration in fetuses of mammals with the aid of a thermistor and piezocrystal. By changing the electroconductivity in temperature variations in the inspired and

expired air the thermistor permits graphic registration of respiration in fetuses. The movements of the thoracic wall in fetuses may be recorded by means of a piezocrystal producing currents in deformation. Both variants of the advocated method enable us to register the respiration in rabbit fetuses beginning from the moment of its appearance, i.e., from the 20th-22nd day of intrauterine development when the weight of the fetuses does not exceed 3-6 gms.

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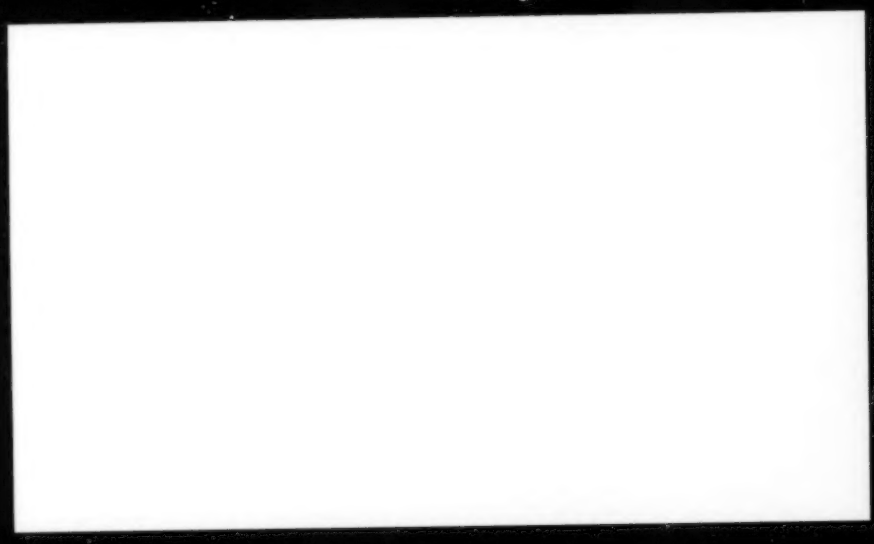

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LÉM	Laboratory for Experimental Morphogenesis
LÉNDVI	Leningrad Inst. of Dermatology and Venereology
LÉO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIÉM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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(continued)

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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